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# KINESIN-LIKE PROTEINS AND METHODS OF USE

## RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/433,098, filed December 13, 2002, which is incorporated herein by reference in its entirety.

## TECHNICAL FIELD

5           This invention relates to kinesin-like proteins, and more particularly to the kinesin-like protein KIF18A, and related polypeptides.

## BACKGROUND

          Kinesin-like proteins (KLPs) play roles in several aspects of spindle assembly and chromosome segregation as well as in some non-mitotic processes (reviewed in Hirokawa *et al.*,  
10   *Curr. Opin. Cell Biol.*, 10:60-73, 1998). At the molecular level, all the members of this family share homology with the so-called “motor domain” of the kinesin heavy chain, which is a microtubule activated ATPase. The non-motor domain of these proteins is responsible for cargo attachment; a “cargo” can include any one of a variety of different membranous organelles, signal transduction scaffolding systems, and chromosomes. Kinesins use the energy of ATP  
15   hydrolysis to move cargo unidirectionally (toward the plus end) along a microtubule. Thus, kinesins are often called “plus-end” directed motors.

*Drosophila* KLP67A is a plus end-directed motor. Cytological studies have shown that KLP67A is associated with tiny 0.1 micron mitochondria at the plus-ends of the astral spindle fibers (Pereira *et al.*, *J. Cell Biol.*, 136:1081-1090, 1997). A human ortholog of KLP67A is  
20   KIF18A (Miki *et al.*, *Proc. Natl. Acad. Sci., USA*, 98:7004-11, 2001).

## SUMMARY

          The kinesin-like genes *KIF18A* (in human) and *KLP67A* (in *Drosophila*) have been found to be involved in processes relating to cell division. Methods and compounds for treating a subject, such as a mammal (*e.g.*, a human), having a proliferative disorder are described herein.  
25   The new methods and compounds target the activity of the human kinesin-like gene *KIF18A*, and homologs and orthologs thereof, including the *Drosophila* ortholog, *KLP67A*.

New methods are described for identifying compounds that modulate the activity of a KIF18A or KLP67A polypeptide. One exemplary method includes a) obtaining a test cell containing a KIF18A or KLP67A polypeptide and a control cell containing a KIF18A or KLP67A polypeptide; b) incubating the test cell with a test compound; and c) detecting an altered localization of the KIF18A or KLP67A polypeptide in the test cell as compared to the KIF18A or KLP67 polypeptide in the control cell, wherein an altered localization indicates that the test compound modulates activity of the KIF18A or KLP67A polypeptide. For example, the KIF18A polypeptide can have the sequence of GenBank Accession Number AL136819 (SEQ ID NO:2), and the KLP67A polypeptide can have the sequence of GenBank Accession Number NM\_079268 (SEQ ID NO:3). In this method, the test compound can be an antisense nucleic acid molecule, a small inhibitory RNA (siRNA), a ribozyme, a triple helix molecule, an antibody, a polypeptide, a peptoid, a polypeptide mimetic, a small inorganic molecule, or a small non-nucleic acid organic molecule.

In certain embodiments, the polypeptide can be localized to a region of a dividing cell other than the distal ends of astral microtubules in the presence of the test compound, and the polypeptide can be localized using immunocytochemistry. In certain embodiments, the polypeptide can be fused to a reporter molecule, such as green fluorescent protein (GFP),  $\beta$ -glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), horseradish peroxidase (HRP), or  $\beta$ -galactosidase.

In another aspect, the invention features methods for identifying a compound that modulates expression of a KIF18A or KLP67A DNA sequence, by a) providing a test cell comprising a nucleic acid that expresses the KIF18A or KLP67A polypeptide and a control cell containing a nucleic acid that expresses the KIF18A or KLP67A polypeptide; b) incubating the test cell with a test compound; and c) detecting an increase or decrease in a KIF18A or KLP67A RNA or polypeptide population as compared to a KIF18A or KLP67A RNA or polypeptide population in a control cell; wherein an increase or decrease in the KIF18A or KLP67A RNA population indicates that expression of the KIF18A or KLP67A DNA is modulated by the test compound. In these methods, the increase or decrease in the RNA population can be assayed by Northern blot, RT-PCR, or microarray analysis, and the increase or decrease in the KIF18A or KLP67A polypeptide population can be assayed by Western blot or ELISA.

The invention also provides various methods of assaying for modulation of activity (or expression) of a KIF18A polypeptide in a test cell. Some of these methods include (a) providing a dividing test cell containing a KIF18A polypeptide (or nucleic acid) and a dividing control cell containing a KIF18A polypeptide (or nucleic acid); (b) measuring spindle length in the dividing  
5 test cell and the dividing control cell; and (c) determining either (i) the amount of KIF18A polypeptide in the test cell and the control cell, or (ii) the location of KIF18A polypeptide in the test cell and the control cell, or both (i) and (ii); wherein the occurrence of a longer or shorter spindle in the test cell as compared to the control cell, and either (i) the amount of KIF18A polypeptide is different than the amount of KIF18A polypeptide in the control cell, or (ii) the  
10 location of KIF18A polypeptide in the test cell is different than the location of KIF18A polypeptide in the control cell, or both (i) and (ii), is an indication that the activity of the KIF18A polypeptide in the test cell is different than the activity of a KIF18A polypeptide in the control cell. For example, the spindle length of the test cell can be increased or decreased by 45-100%, as compared to the spindle length of the control cell.

15 In these methods, one can further determine whether a KIF18A polypeptide from the test cell contains an altered amino acid compared to a wild type KIF18A polypeptide.

In another version of the methods for assaying for modulation of activity of a KIF18A polypeptide, the methods include (a) providing a dividing test cell containing a KIF18A polypeptide and a dividing control cell containing a KIF18A polypeptide; (b) measuring the  
20 angle between two ectopically localized prophase centrosomes in the dividing test cell; and (c) determining either (i) the amount of KIF18A polypeptide in the test cell and the control cell, or (ii) the location of KIF18A polypeptide in the test cell and in the control cell, or both (i) and (ii); wherein the occurrence of a 1-155° angle between the two prophase centrosomes in the dividing cell, and either (i) the amount of KIF18A polypeptide is different than the amount of KIF18A  
25 polypeptide in the control cell, or (ii) the location of KIF18A polypeptide in the test cell is different than the location of KIF18A polypeptide in the control cell, or both (i) and (ii), indicates that the activity of the KIF18A polypeptide in the test cell is different than the activity of a KIF18A polypeptide in the control cell.

30 In these methods, the angle between the two prophase centrosomes in the dividing test cell can range from 130-154°, and the centrosomes can be localized by using an anti-centrosomin antibody and immunocytochemistry.

Another method includes a) providing a dividing test cell containing a KIF18A polypeptide and a dividing control cell containing a KIF18A polypeptide; (b) determining the shape of a spindle or astral microtubule in the dividing test cell and the dividing control cell; and (c) determining either (i) the amount of KIF18A polypeptide in the test cell and the control cell, or (ii) the location of KIF18A polypeptide in the test cell and the control cell, or both (i) and (ii); wherein the occurrence of a spindle or astral microtubule in the dividing test cell that is shaped differently than a spindle or astral microtubule in the control test cell, and either (i) the amount of KIF18A polypeptide is different than the amount of KIF18A polypeptide in the control cell, or (ii) the location of KIF18A polypeptide in the test cell is different than the location of KIF18A polypeptide in the control cell, or both (i) and (ii), indicates that the activity of the KIF18A polypeptide in the test cell is different than the activity of a KIF18A polypeptide in the control cell. In this method, the spindle or astral microtubule in the dividing test cell can be banana-shaped, and the spindle or astral microtubule can be detected using an anti- $\alpha$  tubulin antibody and immunocytochemistry.

Methods for assaying for modulation of expression of a KIF18A nucleic acid include a) providing a test cell containing a KIF18A nucleic acid and a control cell containing a KIF18A nucleic acid; and (b) determining a level of an RNA encoded by the KIF18A nucleic acid in the test cell and in the control cell; wherein an increase or decrease in the level of RNA encoded by the KIF18A nucleic acid in the test cell compared to the level of RNA encoded by the KIF18A nucleic acid in the control cell indicates that the expression of a KIF18A nucleic acid is modulated. For example, the level of RNA can be monitored by Northern blot, RT-PCR, or microarray analysis, and the methods can further include determining whether the KIF18A nucleic acid from the test cell contains a mutation.

In another aspect, the invention features methods for assaying for modulation of expression of a KIF18A nucleic acid by a) providing a test cell containing a KIF18A nucleic acid and a control cell containing a KIF18A nucleic acid; and b) determining a level of a KIF18A polypeptide encoded by the KIF18A nucleic acid in the test cell and in the control cell; wherein an increase or decrease in the level of KIF18A polypeptide encoded by the KIF18A nucleic acid in the test cell compared to the level of polypeptide encoded by the KIF18A nucleic acid in the control cell indicates that expression of the KIF18A nucleic acid is modulated. For example, the level of KIF18A polypeptide in the test cell and in the control cell can be determined by Western

blot or ELISA, and the methods can further include determining whether the KIF18A nucleic acid in the test cell contains a mutation.

The invention also features methods for modulating the activity of a KIF18A polypeptide or a KLP67A polypeptide by a) contacting a KIF18A nucleic acid or KLP67A nucleic acid with a modulating agent in a concentration sufficient to modulate transcription of the nucleic acid; b) contacting a cell expressing a KIF18A nucleic acid or KLP67A nucleic acid with a modulating agent in a concentration sufficient to modulate translation from an RNA encoded by the nucleic acid; or c) contacting a cell expressing the KIF18A polypeptide or KLP67A polypeptide with a compound that binds to the polypeptide in a concentration sufficient to modulate the activity of the polypeptide. In these methods, the modulating agent can be an antisense nucleic acid molecule, a small inhibitory RNA (siRNA), a ribozyme, a triple helix molecule, an antibody, a small inorganic molecule, or a small non-nucleic acid organic molecule.

In another aspect, the invention includes compositions including a compound that modulates the activity of a KIF18A polypeptide, wherein the composition comprises an antisense nucleic acid molecule, an siRNA, a ribozyme, a triple helix molecule, an antibody, a small inorganic molecule, or a small non-nucleic acid organic molecule. For example, the antisense nucleic acid molecule can be complementary to a segment of contiguous nucleotides of a KIF18A nucleotide sequence ranging from a length of 10 to 1000 nucleotides, and the siRNA can include the sequence of SEQ ID NO:14, or a fragment thereof. The antibody or small molecule can specifically bind to a KIF18A polypeptide, or a fragment or an allelic variant thereof.

The invention also includes kits that include the new compositions and instructions to treat a disorder mediated by or associated with a KIF18A polypeptide, such as proliferative disorders, such as cancer or psoriasis, or autoimmune disorders, e.g., rheumatoid arthritis.

“Compounds” or “agents” described herein can modulate the expression or activity of a mammalian KIF18A or Drosophila KLP67A nucleic acid or polypeptide. The new compounds can be, e.g., polypeptides, peptidomimetics, peptoids, small inorganic molecules, small non-nucleic acid organic molecules, and nucleic acid molecules, such as antisense nucleic acid, siRNAs, ribozymes, or triple helix nucleic acids. A “test compound” refers to a compound used in an assay, such as a screening assay, to determine whether or not the compound can modulate KIF18A or KLP67A expression or activity. A test compound that is found to affect KIF18A or

KLP67A expression or activity is called a “candidate compound.” For example, a compound that disrupts spindle formation in a mitotic cell would be a candidate compound. A therapeutic agent can be identified by an assay (e.g., an *in vivo* assay) that tests a “candidate compound.” In other words, a test compound can be a candidate compound, which can be a therapeutic agent.

5           The test compound (or candidate compound or therapeutic agent) can be an antisense nucleic acid molecule, a small inhibitory RNA (siRNA), a ribozyme, a triple helix molecule, an antibody, a polypeptide, a peptoid, a polypeptide mimetic, a small inorganic molecule, or a small non-nucleic acid organic molecule.

          A KIF18A or KLP67A polypeptide can be localized by immunocytochemistry.  
10       Alternatively, or in addition, the polypeptide can be fused to a reporter polypeptide, such as green fluorescent protein (GFP),  $\beta$ -glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), horseradish peroxidase (HRP), or  $\beta$ -galactosidase, and the polypeptide can be localized by the activity of the reporter molecule. In an immunocytochemical technique, a first antibody, which binds the KIF18A or KLP67A polypeptide, can be labeled. Alternatively, a  
15       labeled second antibody (an anti-IgG) can be directed against the first (the primary) antibody. This second antibody can be conjugated with a fluorochrome, an enzyme that can produce a colorimetric reaction, or gold beads (for electron microscopy) so that the location of the primary antibody, and thus the antigen, can be recognized.

          New isolated antibodies that specifically bind to KIF18A polypeptides or fragments  
20       thereof are also described. For example, the KIF18A polypeptide can have the amino acid sequence of SEQ ID NO:2 (FIG. 17), or an allelic variant or fragment thereof. For example, the polypeptide or fragment can consist of amino acid residues 492-507, 492-517, 616-631, 660-667, 730-768, 776-797, or 360-898 of SEQ ID NO:2.

          A polynucleotide or polypeptide that is substantially identical in sequence to a human  
25       *KIF18A* cDNA (SEQ ID NO:1) (FIG. 16) or polypeptide sequence (SEQ ID NO:2) (FIG. 17) is at least 80% identical to SEQ ID NO:1 or SEQ ID NO:2. For example, a target DNA or RNA sequence can be 80%, 85%, 95%, or 100% identical to SEQ ID NO:1. A fragment of a target nucleotide sequence (e.g., a sequence that encodes an exon) can be at least 80% identical to a fragment of SEQ ID NO:1.

30       The invention also features methods of treating a subject having a proliferative disorder associated with aberrant KIF18A polypeptide expression or activity. One such method includes

administering to the subject a therapeutically effective amount of a compound that modulates expression or activity of a KIF18A polypeptide. For example, the compound can be an antisense nucleic acid molecule, an siRNA, a ribozyme, a triple helix molecule, an antibody, a small inorganic molecule, or a small non-nucleic acid organic molecule. The compound can be provided in a kit, which can include instructions for use.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the detailed description, drawings, and from the claims.

## DESCRIPTION OF DRAWINGS

FIG. 1 is a photomicrograph of a primary spermatocyte isolated from the testes of wildtype *Drosophila*, and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The spermatocyte is in the prometaphase I stage of meiosis. The image shows two well-separated prominent asters closely apposed to the nuclear envelope.

FIG. 2 is a photomicrograph showing two prophase I spermatocytes from the testes of *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutants and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The asters are ectopically positioned near one another and disassociated from the nuclear envelope.

FIG. 3 is a photomicrograph showing a prophase I spermatocyte from the testes of the *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutant and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The bulk of one of the centrosomes and its associated aster are detached from the spindle pole.

FIG. 4 is a photomicrograph showing a tetraploid spermatocyte from the testes of the *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutant and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The mutant spermatocyte has four ectopically positioned asters, indicative of failed cytokinesis in a previous gonial division.



FIG. 5 is a photomicrograph of a spermatocyte isolated from the testes of wildtype *Drosophila* and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The spermatocyte is in the metaphase I stage of meiosis.

FIG. 6 is a photomicrograph of a spermatocyte isolated from the testes of wildtype *Drosophila* and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The spermatocyte is in the anaphase I stage of meiosis.

FIG. 7 is a photomicrograph of two spermatocytes isolated from the testes of the *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutant and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The metaphase I spermatocyte (left; arrowhead) has a bipolar spindle. The anaphase I spermatocyte (right; arrow) demonstrated abnormal chromosome segregation. In both cells, the microtubules appear longer than in wildtype spermatocytes (see Figs. 5, 6, 8, 10).

FIG. 8 is a photomicrograph of two spermatocytes isolated from the testes of wildtype *Drosophila* and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The spermatocytes are in the telophase I stage of meiosis.

FIG. 9 is a photomicrograph of three spermatocytes isolated from the testes of the *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutant and stained for  $\alpha$ -tubulin, centrosomin, and DNA. These telophase I spermatocytes demonstrate abnormally long astral microtubules and abnormal central spindles.

FIG. 10 is a photomicrograph of two spermatocytes isolated from the testes of wildtype *Drosophila* and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The spermatocytes are in the telophase II stage of meiosis.

FIG. 11 is a photomicrograph of telophase II spermatocytes isolated from the testes of the *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutant and stained for  $\alpha$ -tubulin, centrosomin, and DNA. The arrowhead points to a morphologically abnormal telophase figure in which interpolar microtubules overlap in the middle of the cell but do not form a typical dense central spindle. The arrow points to two telophases within the same cytoplasm showing long astral microtubules. Generally, the astral microtubules emanating from different spindles overlap in an abnormal fashion.

FIG. 12 is a photomicrograph representing the real time analysis of mitosis in blastoderm embryos of *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutants. Arrows draw attention to the

correlation between incomplete centrosome separation and curved banana-shaped spindles (see FIG. 13).

FIG. 13 is a photomicrograph representing the real time analysis of mitosis in blastoderm embryos of *Drosophila* *Df(3L)29A6/KLP67A<sup>322b24</sup>* mutants. Arrows draw attention to the correlation between curved banana-shaped spindles and incomplete centrosome separation (see FIG. 12).

FIG. 14 is a photomicrograph representing the real time analysis of mitosis in blastoderm embryos of wildtype *Drosophila*. Arrows draw attention to the correlation between incomplete centrosome separation and curved banana-shaped spindles (see FIG. 15). The frequency of incomplete separation in wildtype embryos is less than that observed in *Df(3L)29A6/KLP67A<sup>322b24</sup>* embryos.

FIG. 15 is a photomicrograph representing a real time analysis of mitosis in blastoderm embryos of wildtype *Drosophila*. Arrows draw attention to the correlation between curved banana-shaped spindles and incomplete centrosome separation (see FIG. 14). The frequency of incomplete separation in wildtype embryos is less than that observed in *Df(3L)29A6/KLP67A<sup>322b24</sup>* embryos.

FIG. 16 is the nucleotide sequence of a human KIF18A cDNA (SEQ ID NO:1) (GenBank Accession No. AL136819).

FIG. 17 is the amino acid sequence of a human KIF18A polypeptide (SEQ ID NO:2) (GenBank Accession No. AL136819).

FIG. 18 is the amino acid sequence of a KLP67A polypeptide (SEQ ID NO:3) from *Drosophila melanogaster* (GenBank Accession No. NM\_079268).

FIG. 19 is the nucleotide sequence of a KLP67A cDNA (SEQ ID NO:4) from *Drosophila melanogaster* (GenBank Accession No. NM\_079268).

FIG. 20 is the nucleotide sequence of a dsRNA targeting KLP61F nucleic acid (SEQ ID NO:13) in *Drosophila melanogaster*.

FIG. 21 is the nucleotide sequence of a dsRNA targeting KLP67A nucleic acid (SEQ ID NO:14) in *Drosophila melanogaster*.

## DETAILED DESCRIPTION

The invention relates to compounds that can modulate cell proliferation, and to methods of identifying and using such compounds. Compounds capable of modulating cell proliferation can be useful for treating a subject having a proliferative disorder such as cancer. The subject  
 5 can be an animal, such as a mammal, or a human. Generally, the methods and compounds target the activity of the kinesin-like protein KIF18A and homologs thereof, and a gene encoding *KIF18A* or a substantially identical gene. Cell division can be modulated by altering the expression or activity of a *KIF18A* gene or a KIF18A polypeptide. More specifically, the proper assembly, stabilization, and function of the spindle during mitosis is modulated, *e.g.*, inhibited or  
 10 enhanced, to inhibit or enhance the process of cell division in specific cells.

KIF18A is a “kinesin superfamily” (KIF) protein. Members of the KIF protein family are known to participate in chromosomal and spindle movements during mitosis and meiosis (Hirokawa *et al.*, *Curr. Opin. Cell Biol.*, 10:60-73, 1998; Vale and Fletterick, *Annu. Rev. Cell Dev. Biol.*, 13:745-777, 1997; Sharp *et al.*, *Nature*, 407:41-47, 2000). The term homolog can  
 15 refer to a nucleic acid variant of *KIF18A*. An orthologous gene is related by phylogenetic descent but is found in a different organism. Orthologous genes share functional homology, although not necessarily a strong sequence homology. The amino acid sequences that define the motor domains of human KIF18A and *Drosophila* KLP67A are 52% identical and 69% similar. Thus, as defined herein, a KIF18A ortholog can have an amino acid sequence that defines a  
 20 functional domain, *e.g.*, a motor domain or a cargo domain, that is at least 65% similar, 67% similar, or 69% similar to the corresponding KIF18A sequence.

The methods and compounds described herein can target the activity of a nucleic acid, *e.g.*, a DNA or mRNA having the sequence of SEQ ID NO:1 (the nucleotide sequence of the human *KIF18A* cDNA) (FIG. 16) or any substantially identical sequence or any fragment thereof.  
 25 For example, a composition can target a nucleic acid, such as a gene, that encodes a cDNA substantially identical to SEQ ID NO:1 (FIG. 16). A target DNA sequence can be a variant nucleic acid that differs from the wildtype sequence. Variants can be naturally occurring, such as allelic variants (*i.e.*, variants located at the same genetic locus); substantially identical sequences, such as those that occur at different loci; and orthologs. Alternatively, variants can be non-  
 30 naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. Variants can contain nucleotide

substitutions, deletions, inversions and/or insertions. Variation can occur in either or both the coding and non-coding regions. A variant nucleotide sequence can encode a conservative or non-conservative amino acid substitution (as compared to the wildtype encoded product). By its sequence similarity, particularly in the motor domain, and its similar intracellular localization pattern as described herein, the *Drosophila* gene *KLP67A* is an ortholog of human *KIF18A*. Thus, the methods and compounds described herein can target the activity of a nucleotide or polypeptide sequence of KLP67A. For example, the methods can target a nucleotide sequence consisting of SEQ ID NO:4 (FIG. 19) or an amino acid sequence consisting of SEQ ID NO:3 (FIG. 24). Variants of KIF18A are referred to herein as KIF18A-related polypeptides or nucleic acids.

To determine the percent identity of two amino acid sequences, or two nucleotide sequences, the sequences can be aligned for optimal comparison purposes. For example, gaps can be introduced in one or both of a first and a second amino acid or nucleotide sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes. The length of a reference sequence aligned for comparison purposes can be at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, or 100% of the length of the reference sequence. For example, when aligning a second sequence to the KIF18A amino acid sequence of SEQ ID NO:2 (FIG. 17) having 899 amino acid residues, at least 270, preferably at least 360, more preferably at least 450, even more preferably at least 540, and even more preferably at least 630, 720, or 810 amino acid residues are aligned. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions can then be compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The determination of percent identity between two amino acid sequences can be accomplished using the BLAST 2.0 program (Gish, W., personal communication). Sequence comparison is performed using an ungapped alignment and using the default parameters (Blossom 62 matrix, gap existence cost of 11, per residue gapped cost of 1, and a lambda ratio of 0.85). The mathematical algorithm used in BLAST programs is described in Altschul *et al.* (*Nucleic Acids Res.*, 25:3389-3402, 1997).

The methods and compounds can target a polypeptide having the amino acid sequence of SEQ ID NO:2 (FIG. 17), which is the amino acid sequence of the human KIF18A polypeptide, any substantially identical sequence, or any fragment thereof.

The compounds used to modulate KIF18A expression or activity can be one or more of an siRNA, antisense nucleic acid molecule, ribozyme, triple helix molecule, antibody, or small molecule. For example, an siRNA, antisense nucleic acid molecule, or ribozyme targeted to a *KIF18A* RNA can promote the degradation of an RNA expressed from a gene having the sequence of SEQ ID NO:1 (FIG. 16) or a substantially identical sequence. A triple helix molecule can be used to inhibit expression of a *KIF18A* target gene by interacting with DNA. An antibody or small molecule that can interact with a KIF18A polypeptide (e.g., SEQ ID NO:2 (FIG. 17), or a substantially identical sequence or fragment thereof) can be used to modulate polypeptide activity, such as to inhibit or increase activity. If KIF18A expression or activity is down regulated as a result of contact with one of the described compounds, spindle assembly and function during mitosis can be inhibited. Cell division would therefore be inhibited, completely or partially.

According to one method described herein, a nucleic acid molecule that encodes and expresses a wildtype or modified (e.g., engineered) KIF18A polypeptide can be introduced into cell that contains a mutant allele of a *KIF18A* nucleic acid. The nucleic acid molecule can be introduced into the cell via a gene therapy method.

### Screening Assays

Methods are described for identifying compounds (e.g., polypeptides, peptidomimetics, peptoids, small inorganic molecules, small non-nucleic acid organic molecules, nucleic acid molecules such as antisense nucleic acids, siRNAs, ribozymes, or triple helix molecules, or other drugs) that modulate KIF18A gene expression or protein activity, or expression or activity of a KIF18A ortholog, such as *Drosophila* KLP67A. These methods are also referred to herein as “screening assays.” Compounds thus identified can have a stimulatory or inhibitory effect on KIF18A expression or activity, or can have a stimulatory or inhibitory effect on the expression or activity of a KIF18A substrate. The compounds can be used to modulate the expression or activity of KIF18A or disrupt normal KIF18A interactions. Compounds that affect KIF18A expression or activity can be useful in therapeutic protocols, such as to inhibit undesirable

cellular proliferation as occurs, for example, in cancer or psoriasis. Methods described herein can also be used to identify modulators that bind to or alter the activity of a *Drosophila KLP67A* polypeptide or nucleic acid. A compound that can modulate expression or activity of a KIF18A gene or polypeptide or a KLP67A gene or polypeptide is referred to as a modulating agent.

5           Assays for screening candidate or test compounds that bind to and/or modulate the activity of a KIF18A polypeptide or a biologically active portion thereof are also described. In such assays, the KIF18A polypeptide typically has a motor domain and/or cargo domain. For example, the KIF18A motor domain is from about amino acids 8-283 of SEQ ID NO:2 (FIG. 17).

10           The test compounds used in the screening assays described herein can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including but not limited to: biological libraries; peptoid libraries (libraries of molecules having the functionalities of polypeptides, but with a novel, non-polypeptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckermann *et al.*, *J. Med. Chem.*, 37:2678-85, 1994); spatially addressable parallel solid phase or solution phase  
15           libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-polypeptide oligomer or small molecule libraries of compounds (Lam, *Anticancer Drug Des.*, 12:145, 1997).

20           Examples of methods for the synthesis of molecular libraries can be found in the art. For example, see DeWitt *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6909, 1993; Erb *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:11422, 1994; Zuckermann *et al.*, *J. Med. Chem.*, 37:2678, 1994; Cho *et al.*, *Science*, 261:1303, 1993; Carrell *et al.*, *Angew. Chem. Int. Ed. Engl.*, 33:2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.*, 33:2061, 1994; and Gallop *et al.*, *J. Med. Chem.*, 37:1233, 1994.

25           Libraries of compounds can be presented in solution (*e.g.*, Houghten, *Biotechniques*, 13:412-421, 1992), or on beads (Lam, *Nature*, 354:82-84, 1991), chips (Fodor, *Nature*, 364:555-556, 1993), bacteria or spores (Ladner, U.S. Patent No. 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci., USA*, 89:1865-1869, 1992) or on phage (Scott and Smith, *Science*, 249:386-390, 1990; Devlin, *Science*, 249:404-406, 1990; Cwirla *et al.*, *Proc. Natl. Acad. Sci., USA*, 87:6378-6382, 1990; Felici, *J. Mol. Biol.*, 222:301-310, 1991; and Ladner, *supra*).

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An assay can be, for example, a cell-based assay in which a cell that expresses a KIF18A polypeptide or biologically active portion thereof is contacted with a test compound. The contact is typically maintained for the time necessary for an untreated cell to progress through at least one cell cycle. The ability of the test compound to modulate KIF18A expression or activity is then determined. Examples of tests for KIF18A expression or activity are described below. The cell, for example, can be of mammalian origin, such as from human, mouse, rat, hamster, or monkey.

KIF18A localization assays can be employed to evaluate altered KIF18A function in the presence of a test compound. Under wildtype conditions, KIF18A localizes to the distal ends of the astral microtubules and extends into the growth cone of interphase cells. Localization can be achieved, for example, by using immunocytochemistry with antibodies that specifically bind to a KIF18A polypeptide, or by transfecting a cell with a reporter gene fused to a sequence encoding a KIF18A polypeptide. Exemplary reporter genes include green fluorescent protein (GFP),  $\beta$ -glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), horseradish peroxidase (HRP), and  $\beta$ -galactosidase. Methods of producing such fusion polypeptides are known in the art.

To perform a localization assay, a transfected cell can be incubated under conditions appropriate for expression of a *KIF18A* fusion gene. For example, to assay a test compound, the cell can be contacted with the test compound, and localization of the fusion polypeptide in the presence and absence of the test compound can be determined. An altered localization of the fusion polypeptide in the presence of the test compound indicates that the test compound modulates KIF18A expression or activity either directly or indirectly. In such assays, it is also generally necessary to demonstrate that the fusion polypeptide co-localizes with wildtype KIF18A.

The ability of the test compound to modulate KIF18A expression or activity can also be assayed by monitoring spindle assembly during mitosis of a test cell, such as by fluorescence microscopy. The architecture of the mitotic spindle is indicative of additional, often more subtle, cellular phenotypes caused by modulation of KIF18A activity. For example, inhibition of KIF18A can result in decreased microtubule depolymerization and consequently to increased microtubule length during mitosis and meiosis (see Examples 3 and 4; compare Figs. 6 and 7, and 8 and 9). Conversely, KIF18A hyperactivity can lead to increased microtubule

depolymerization. Altered rates of microtubule depolymerization are manifested by modifications in the length of the mitotic spindle. For example, a decreased rate of microtubule depolymerization can lead to an increase in spindle length by 45-200%, typically 50-100%. Methods to monitor spindle morphology are described below and are known in the art.

5           Inhibition of KIF18A can result in a variety of other subcellular phenotypes including perturbations in centrosome separation, chromosome segregation and central spindle assembly, which all affect cell division. The presence of two daughter nuclei of different sizes can be an indication of aberrant chromosome segregation (see Example 3). KIF18A deficient cells also exhibit abnormally curved microtubules. The spindles of cells in which KIF18A activity is  
10           inhibited are generally curved in appearance, *e.g.*, banana-shaped (see Example 4, compare FIGs. 12 and 13). Thus, introduction of a test compound into a cell culture, and incubation for a suitable period of time (such as the time required for at least one cell cycle in the absence of the test compound), followed by fixation and detection of microtubules can be used to confirm whether a test compound that affects KIF18A can affect cell division.

15           Another phenotype attributed to KIF18A inhibition is the ectopic localization of prophase centrosomes in the cytoplasm, *e.g.*, at an angle less than 155.0° from each other, such as at an angle ranging from 1-155°, from 20-155°, from 100-155°, or 130-154° from each other. During normal cell division, cells are typically located further apart, ranging from 155°-180° apart (*e.g.*, from 160°-170° apart) (see Example 4, FIGs. 12 and 13). Centrosomes and their associated  
20           asters can also appear to detach from the spindle poles, giving rise to asters that freely float in the cytoplasm. The localization of the asters, which mark the centrosomes, can be accomplished by immunocytochemistry experiments that include exposing the cell to an anti-centrosomin antibody. Generally, the timing of centrosome separation is also increased in KIF18A deficient cells. The period of centrosome migration up until the point of nuclear envelope breakdown can  
25           be increased by 100-600% (*e.g.*, 200, 300, or 400%).

          Cell structure morphologies can be monitored using a combination of immunocytochemistry and specialized stains. For example, cell structure morphology can be assayed by labeling with anti- $\alpha$  tubulin (Sigma; St. Louis, MO), anti-centrosomin, and/or anti-myosin II antibodies. Actin staining, such as with a phalloidin (*e.g.*, rhodamine-phalloidin;  
30           Molecular Probes; Eugene, OR) can also be used to examine cell morphology. A fluorescent



conjugate of tubulin, such as a tetramethylrhodamine-tubulin; Cytoskeleton Inc., Denver, CO) can be injected into cells to generate fluorescent microtubules for assay *in vivo*.

The ability of a test compound to modulate KIF18A binding to a cellular molecule, such as a microtubule, can also be evaluated. This can be accomplished *in vivo* or *in vitro*, for example, by coupling the molecule with a radioisotope or enzymatic label such that binding of the molecule to KIF18A can be determined by detecting the labeled molecule in a complex. For example, molecules can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, molecules can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

In one alternative, a cell-free assay can be provided in which a KIF18A polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the KIF18A polypeptide or biologically active portion thereof is evaluated. The biologically active portions of the KIF18A polypeptides to be used in assays described herein can include fragments that participate in interactions with non-KIF18A molecules.

KIF18A polypeptides or biologically active portions thereof can be used in the cell-free assays described herein. Cell-free assays involve preparing a reaction mixture of KIF18A and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be displaced and/or detected.

The interaction between two molecules (*e.g.*, a KIF18A polypeptide or fragment thereof, and a test compound) can also be detected, for example, by using Fluorescence Resonant Energy Transfer (FRET). In this example, a fluorophore label on the first, 'donor' molecule (*e.g.*, a KIF18A polypeptide or biologically active portion) is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule (*e.g.*, a test compound), which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' polypeptide molecule can utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label can be differentiated from that of the 'donor.' Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the

fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. A FRET binding event can be measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

The ability of a KIF18A polypeptide to bind to a target molecule can also be determined using real-time Biomolecular Interaction Analysis (BIA) (see, *e.g.*, Sjolander and Urbaniczky, *Anal. Chem.*, 63:2338-2345, 1991; and Szabo *et al.*, *Curr. Opin. Struct. Biol.*, 5:699-705, 1995). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules. The target molecule can be a test compound. To assay the ability of a test compound to modulate the interaction between KIF18A and a KIF18A substrate such as a microtubule, the incubation mix can contain a KIF18A polypeptide or fragment thereof, a KIF18A substrate, and the test compound. An alteration in the interaction between the KIF18A polypeptide or fragment and the substrate in the presence of the test compound indicates that the test compound is a candidate compound for modulating KIF18A activity.

In one example, a KIF18A polypeptide or a test compound is anchored onto a solid phase. The KIF18A/test compound complexes anchored on the solid phase can be detected at the end of the reaction. For example, KIF18A can be anchored onto a solid surface, and the test compound (which is not anchored) can be labeled, either directly or indirectly, with detectable labels, some of which are discussed herein.

A KIF18A polypeptide, an anti-KIF18A antibody; or a KIF18A target molecule (*e.g.*, a binding partner, such as a microtubule) can be immobilized to facilitate separation of complexed from uncomplexed forms of one or both of the polypeptides, as well as to accommodate automation of the assay. Binding of a test compound to a KIF18A polypeptide, or interaction of a KIF18A polypeptide with a target molecule in the presence and absence of a test compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion polypeptide can be provided that adds a domain that allows one or both of the polypeptides to be bound to a matrix. For example, a glutathione-S-transferase/ KIF18A fusion

polypeptides or glutathione-S-transferase/target fusion polypeptides can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound, or the test compound and either the non-adsorbed target polypeptide or KIF18A polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells can be washed to remove any unbound components, the matrix immobilized in the case of beads, and the complex assayed either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix and the level of KIF18A binding or activity determined using methods known in the art.

Other techniques for immobilizing either a KIF18A polypeptide or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated KIF18A polypeptide or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). To conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, such as by using a labeled antibody-specific for the non-immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, for example, a labeled anti-Ig antibody). To assay the ability of a test compound to bind to a KIF18A polypeptide or fragment thereof, either the test compound or the KIF18A polypeptide or fragment can be the immobilized component. The components can be, for example, a KIF18A polypeptide or fragment thereof, and a KIF18A substrate, such as a microtubule. The assay can be performed by comparing binding of the KIF18A polypeptide or fragment to the substrate in the presence and absence of the test compound. An alteration in the amount of binding in the

presence of the test compound indicates that the test compound is a candidate compound for modulating KIF18A activity.

The assay can be performed by utilizing antibodies that specifically bind to KIF18A polypeptide or target molecules, but that do not interfere with binding of the KIF18A polypeptide to its target molecule (*e.g.*, the microtubule). Such antibodies can be derivatized to the wells of the plate, and unbound target or KIF18A polypeptide can be trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the KIF18A polypeptide or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the KIF18A polypeptide or target molecule.

Cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci.*, 18:284-7, 1993); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, *e.g.*, Ausubel *et al.*, eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel *et al.*, *supra*). Such resins and chromatographic techniques are known to one skilled in the art (see, *e.g.*, Heegaard, *J. Mol. Recognit.* 11:141-8, 1998; Hage and Tweed, *J. Chromatogr. B. Biomed. Sci. Appl.* 699:499-525, 1997). Further, fluorescence energy transfer can also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

A cell-free assay can include contacting the KIF18A polypeptide or biologically active portion thereof with a known compound which binds KIF18A to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a KIF18A polypeptide, wherein determining the ability of the test compound to interact with a KIF18A polypeptide includes determining the ability of the test compound to preferentially bind to KIF18A or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

Compounds that disrupt binding of KIF18A to a cellular molecule (*i.e.*, a binding partner), such as a microtubule, can be useful for regulating the activity of KIF18A. Such compounds can include, but are not limited to antibodies, polypeptides, peptoids,

peptidomimetics, small non-nucleic acid organic molecules, and small inorganic molecules. In one alternative, or optionally, the ability of the test compound to modulate the activity of a KIF18A polypeptide can be tested by assay of the activity of a downstream effector of a KIF18A target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between a KIF18A polypeptide, and a cellular binding partner, such as a microtubule, a reaction mixture containing a KIF18A polypeptide and the binding partner is prepared under conditions and for a time sufficient to allow the two products to form a complex. To test an inhibitory compound, the reaction mixture can be provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of KIF18A and its cellular binding partner. Control reaction mixtures can be incubated without the test compound or with a control solution such as buffer that does not contain the test compound. The formation of complexes between KIF18A and the binding partner can then be detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction between KIF18A and the binding partner. Alternatively, or in addition, complex formation within reaction mixtures containing the test compound and normal KIF18A can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases where it is desirable to identify compounds that disrupt interactions of mutant but not normal *KIF18A* gene products. In this and all assays described herein, a fragment of a KIF18A polypeptide can be used.

These assays can be conducted in a heterogeneous or homogeneous format.

Heterogeneous assays involve anchoring either a KIF18A polypeptide or biologically active fragment thereof, or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between KIF18A and a binding partner, such as by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively,

test compounds that disrupt preformed complexes (*e.g.*, compounds with higher binding constants that displace one of the components from the complex) can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

5           In a heterogeneous assay system, either KIF18A or the binding partner can be anchored onto a solid surface, such as a microtiter plate, while the non-anchored species is labeled either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

10           To conduct the assay, the partner of the immobilized species can be exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components can be removed (*e.g.*, by washing), and any complexes that have formed will remain immobilized on the solid surface. In assays where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. In  
15           assays where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, such as by using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, for example, a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed  
20           complexes can be detected.

          Alternatively, or in addition, the reaction can be conducted in a liquid phase in the presence or absence of the test compound. Reaction products can be separated from unreacted components and complexes can be assayed using, for example, a first immobilized antibody specific for one of the binding components to anchor any complexes formed in solution and a  
25           second labeled antibody specific for the other partner to detect anchored complexes. This method or a variation thereof can be used to identify compounds that inhibit complex formation or that disrupt preformed complexes.

          Other homogeneous assays can also be used. For example, a preformed complex of a KIF18A polypeptide or biologically active portion thereof, and the interactive binding partner  
30           product is prepared in which either KIF18A or the binding partner is labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent

No. 4,109,496 that utilizes this approach for immunoassays). The addition of a compound that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt KIF18A-binding partner interaction can be identified.

5           The KIF18A polypeptides can also be used as "bait proteins," such as in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.*, *Cell*, 72:223-232, 1993; Madura *et al.*, *J. Biol. Chem.*, 268:12046-12054, 1993; Bartel *et al.*, *Biotechniques*, 14:920-924, 1993; Iwabuchi *et al.*, *Oncogene*, 8:1693-1696, 1993; and Brent, WO 94/10300), to identify other polypeptides that bind to or interact with KIF18A ("KIF18A -binding  
10 polypeptides" or "KIF18A-bp") and are involved in KIF18A activity. Such KIF18A-bps can be activators or inhibitors of signals by the KIF18A polypeptides or KIF18A targets as, for example, downstream elements of a KIF18A-mediated signaling pathway.

          The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two  
15 different DNA constructs. In one construct, a nucleotide sequence that codes for a KIF18A polypeptide or biologically active portion thereof, is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL4). In the other construct, a DNA sequence from a library of DNA sequences that encodes an unidentified protein ("prey" or "sample") is fused to a nucleotide sequence that codes for the activation domain of the known transcription  
20 factor. Alternatively, the *KIF18A* nucleotide sequence can be fused to the sequence encoding an activator domain. If the "bait" and the "prey" proteins are able to interact *in vivo* and form a KIF18A-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, *LacZ*) that is operably linked to a transcriptional regulatory site responsive to the transcription  
25 factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein that interacts with the KIF18A polypeptide.

          The two hybrid system can be used, for example, to identify modulators of *KIF18A* gene expression. For example, a cell or cell free mixture is contacted with a test compound and the  
30 expression of a KIF18A nucleic acid or polypeptide can be evaluated relative to the level of expression of KIF18A in the absence of the test compound. When expression of a KIF18A

nucleic acid or polypeptide is greater in the presence of the test compound than in its absence, the candidate compound is identified as a stimulator of KIF18A nucleic acid or polypeptide expression. Alternatively, when expression of KIF18A nucleic acid or polypeptide is less (statistically significantly less) in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of KIF18A nucleic acid or polypeptide expression. The level of KIF18A nucleic acid or polypeptide expression can be determined by methods described herein. For example, quantitation of RNA can be accomplished by Northern blot analysis or quantitative RT-PCR. Microarray analysis can also be used to determine whether RNA levels are increased or decreased as compared to wildtype levels after treatment with a test compound. Polypeptide levels can be monitored and/or quantitated by Western blot or ELISA.

Any two or more of the assays described herein can be performed sequentially or in combination. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the compound to modulate the activity of a KIF18A polypeptide can be confirmed *in vivo*, such as in mitotically dividing cell culture.

Compounds identified by the above-described screening assays also relate to the invention described herein. Accordingly, it is within the scope of this invention to further use a compound identified as described herein (*e.g.*, a KIF18A modulating agent, an antisense *KIF18A* nucleic acid molecule, a KIF18A-specific antibody, or a KIF18A-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such a compound. Furthermore, novel compounds identified by the above-described screening assays can be used for treatments as described herein.

The methods described herein can be used to identify modifiers of KIF18A-related polypeptides or nucleic acids, such as *Drosophila* KLP67A polypeptides or nucleic acids. For example, the methods can be used to identify a compound that alters KLP67A activity, thereby identifying a candidate modulator of KIF18A activity. The identified modulator can then be assayed for a direct or indirect effect on KIF18A activity.

#### Anti-KIF18A Antibodies

Anti-KIF18A antibodies can be made using standard techniques. The term "antibody," as used herein, refers to an immunoglobulin molecule or immunologically active portion thereof (*i.e.*, an antigen-binding portion). Examples of immunologically active portions of



immunoglobulin molecules include Fab and F(ab')<sub>2</sub> fragments that can be generated by treating the antibody with an enzyme such as pepsin.

The antibody can be a polyclonal, monoclonal, recombinant (*e.g.*, a chimeric or humanized, fully human, non-human, such as murine), or single chain antibody. The antibody  
5 can be coupled to a toxin or imaging agent.

An isolated or purified full-length KIF18A polypeptide, or antigenic polypeptide fragment of KIF18A, can be used as an immunogen or can be used to identify anti-KIF18A antibodies made with other immunogens, such as cells, membrane preparations, and the like. Exemplary antigenic polypeptide fragments are defined by amino acid sequence fragments of  
10 SEQ ID NO:2 (FIG. 17), including, for example, residues 492-507, 492-517, 616-631, 660-667, 753-768, 776-797, or 360-898. The antigenic polypeptide will preferably encompass a highly charged epitope of KIF18A.

As used herein, an "isolated" or "purified" KIF18A polypeptide or polypeptide fragment is a KIF18A polypeptide or polypeptide fragment that is substantially free of cellular material or  
15 other contaminating polypeptides from the cell or tissue source in which the KIF18A polypeptide naturally occurs. A KIF18A polypeptide fragment that is substantially free of cellular material includes preparations of KIF18A polypeptide fragments having less than about 30% (by dry weight) of non-KIF18A polypeptide.

KIF18A polypeptide fusion polypeptides include all or part of a KIF18A sequence fused  
20 to sequences derived from a member of the immunoglobulin polypeptide family. The resulting KIF18A-immunoglobulin fusion polypeptide can be used as an immunogen to produce anti-KIF18A antibodies in an animal, to purify KIF18A ligands, and in screening assays to identify molecules that can inhibit the interaction of a KIF18A polypeptide with a substrate, such as a microtubule.

A KIF18A chimeric or fusion polypeptide can be produced by conventional recombinant  
25 DNA techniques. For example, DNA fragments coding for the different polypeptide sequences can be ligated together in-frame in accordance with conventional techniques. This can be done using blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment  
30 to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers.

Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, *e.g.*, Ausubel *et al.* (eds), Current Protocols in Molecular Biology, John Wiley & Sons, 1992).

Moreover, many expression vectors are commercially available that already encode a fusion moiety, such as a GST polypeptide. A KIF18A-encoding nucleic acid can be cloned into such an expression vector such that the sequence encoding the fusion moiety is linked in-frame to the sequence encoding the KIF18A polypeptide.

An isolated KIF18A epitope can be used as an immunogen to generate antibodies that bind a KIF18A polypeptide or epitope using standard techniques for polyclonal and monoclonal antibody preparation. Immunogens that include an antigenic polypeptide that includes all or part of a KIF18A amino acid sequence are particularly related to the invention described herein. The antigenic polypeptide of KIF18A consists of at least 8 (preferably 10, 15, 20, or 30) amino acid residues of the amino acid sequence shown in SEQ ID NO:2 (FIG. 17) such that an antibody raised against the polypeptide forms a specific immune complex with a KIF18A polypeptide or polypeptide fragment.

A KIF18A epitope immunogen is used to prepare antibodies by immunizing a suitable animal, such as a rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed KIF18A or a chemically synthesized KIF18A polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent. Immunization with an immunogenic KIF18A epitope preparation induces a polyclonal anti-KIF18A antibody response.

Examples of immunologically active portions of immunoglobulin molecules include Fab and F(ab')<sub>2</sub> fragments, which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind KIF18A or a KIF18A polypeptide fragment. The term "monoclonal antibody" or "monoclonal antibody composition," as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of KIF18A. A monoclonal antibody composition thus typically displays a single binding affinity for a particular KIF18A epitope with which it immunoreacts.

Polyclonal KIF18A epitope antibodies can be prepared as described above by immunizing a suitable subject with a KIF18A epitope immunogen. The anti-KIF18A epitope antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized KIF18A polypeptide or polypeptide fragment. If desired, the antibody molecules directed against a KIF18A polypeptide fragment can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-KIF18A antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein, *Nature*, 256:495-497, 1975, the human B cell hybridoma technique (Kozbor *et al.*, *Immunol. Today*, 4:72, 1983), the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96, 1985) or trioma techniques. The technology for producing various antibodies monoclonal antibody hybridomas is well known (see generally Coligan *et al.* (eds.) Current Protocols in Immunology, John Wiley & Sons, Inc., New York, NY, 1994).

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a KIF18A epitope monoclonal antibody (see, *e.g.*, Coligan *et al.*, *supra*; Galfre *et al.*, *Nature*, 266:55052, 1977; Kenneth, in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York, 1980; and Lerner, *J. Biol. Med.*, 54:387-402, 1981). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line, *e.g.*, a myeloma cell line that is sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the

fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind a KIF18A epitope, *e.g.*, using a conventional ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-KIF18A antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with a KIF18A polypeptide or polypeptide fragment to thereby isolate immunoglobulin library members that bind a KIF18A polypeptide epitope. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs *et al.*, *Bio/Technology*, 9:1370-1372, 1991; Hay *et al.*, *Hum. Antibod. Hybridomas*, 3:81-85, 1992; Huse *et al.*, *Science*, 246:1275-1281, 1989; Griffiths *et al.*, *EMBO J.*, 12:725-734, 1993.

Additionally, recombinant anti-KIF18A antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better *et al.*, *Science*, 240:1041-1043, 1988; Liu *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:3439-3443, 1987; Liu *et al.*, *J. Immunol.*, 139:3521-3526, 1987; Sun *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:214-218, 1987; Nishimura *et al.*, *Canc. Res.*, 47:999-1005, 1987; Wood *et al.*, *Nature*, 314:446-449, 1985; and Shaw *et al.*, *J. Natl. Cancer Inst.*, 80:1553-1559, 1988; Morrison, *Science*, 229:1202-1207, 1985; Oi *et al.*,

*Bio/Techniques*, 4:214, 1986; U.S. Patent 5,225,539; Jones *et al.*, *Nature*, 321:552-525, 1986; Verhoeyan *et al.*, *Science*, 239:1534, 1988; and Beidler *et al.*, *J. Immunol.*, 141:4053-4060, 1988.

An anti-KIF18A antibody (*e.g.*, monoclonal antibody) is useful for isolating a KIF18A polypeptide or polypeptide fragment by standard techniques, such as affinity chromatography or immunoprecipitation. For example, an anti-KIF18A antibody can be used to facilitate the purification of natural KIF18A from cells and of a recombinantly produced KIF18A polypeptide or polypeptide fragment expressed in host cells. Such antibodies are also useful for detecting a KIF18A polypeptide or polypeptide fragment, such as in a cellular lysate or cell supernatant, in order to evaluate the abundance, pattern of expression, and localization of the KIF18A polypeptide or polypeptide fragment. Anti-KIF18A antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure to, for example, determine the efficacy of a given treatment regimen for spinal muscular atrophy. Antibody detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

Antibodies specific for a KIF18A polypeptide or polypeptide fragment are useful for screening assays as described herein, as commercially available reagents, or for other uses of antibodies that are known in the art.

#### Diagnostic and Prognostic Assays

The presence, level, or absence of a KIF18A polypeptide or nucleic acid in a biological sample, particularly following treatment with a method and/or pharmaceutical composition of the invention, can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or agent capable of detecting a KIF18A polypeptide or

nucleic acid (e.g., mRNA or genomic DNA) such that the presence of a KIF18A polypeptide or nucleic acid is detected in the biological sample. "Biological samples" include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. The level of expression of the *KIF18A* gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the *KIF18A* gene; measuring the amount of polypeptide encoded by the *KIF18A* gene; or measuring the activity of the polypeptide encoded by the *KIF18A* gene.

The level of mRNA corresponding to the *KIF18A* gene in a cell can be determined using any suitable protocol, *in situ* or *in vitro*.

An isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Northern blot analyses, polymerase chain reaction analyses, and probe arrays. One diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length *KIF18A* nucleic acid, such as the nucleic acid of SEQ ID NO:1 (FIG. 16), or an oligonucleotide of at least 7, 15, 30, 50, 100, 250, or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to *KIF18A* mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probe. For example, the isolated mRNA can be separated on an agarose gel and transferred to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, such as in a two-dimensional gene chip array. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA expressed by a *KIF18A* gene.

The level of mRNA in a sample that is encoded by a *KIF18A* nucleic acid can be evaluated with nucleic acid amplification, such as by RT-PCR (Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, Proc. Natl. Acad. Sci. USA, 88:189-193, 1991), self-sustained sequence replication (Guatelli *et al.*, Proc. Natl. Acad. Sci. USA, 87:1874-1878, 1990), transcriptional amplification system (Kwoh *et al.*, Proc. Natl. Acad. Sci. USA, 86:1173-1177, 1989), Q-Beta Replicase (Lizardi *et al.*, Bio/Technology, 6:1197, 1988), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033), or any other nucleic acid amplification method,

followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and that contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the *KIF18A* gene being analyzed.

In another embodiment, the *in situ* methods further consist of contacting a control sample with a compound or agent capable of detecting *KIF18A* mRNA, or genomic DNA, and comparing the presence of *KIF18A* mRNA or genomic DNA in the control sample with the presence of *KIF18A* mRNA or genomic DNA in the test sample.

A variety of methods can be used to determine the level of protein encoded by *KIF18A*. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody, with a sample, to evaluate the level of protein in the sample. The antibody can optionally be conjugated to a detectable label. Antibodies can be polyclonal or monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect KIF18A polypeptide in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of KIF18A polypeptide include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassays (EIA), radioimmunoassays (RIA), and Western blot analysis. *In vivo* techniques for detection of a KIF18A polypeptide include introducing into a subject a labeled anti- KIF18A antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In an alternative method, the control sample and the test sample can be contacted with a compound or agent capable of detecting a KIF18A polypeptide. The presence (*e.g.*, amount) of KIF18A polypeptide in the control sample can be compared to the presence (*e.g.*, amount) of KIF18A polypeptide in the test sample.

5           The invention also includes kits for detecting the presence of a KIF18A polypeptide or nucleic acid in a biological sample. The kits can include (1) a compound or agent capable of detecting a KIF18A polypeptide or nucleic acid in a biological sample, and (2) a standard. The compound or agent can be packaged in a suitable container. The kits can further include instructions for using the kit to detect a KIF18A polypeptide or nucleic acid.

10           For antibody-based kits, the kits can include: (1) a first antibody (*e.g.*, attached to a solid support) which binds to a KIF18A polypeptide, and, optionally, (2) a second antibody that binds to either the polypeptide or to the first antibody and is conjugated to a detectable agent.

          For oligonucleotide-based kits, the kits can include: (1) an oligonucleotide (*e.g.*, a detectably labeled oligonucleotide) that hybridizes to a nucleotide sequence encoding a KIF18A polypeptide or (2) a pair of primers useful for amplifying a *KIF18A* nucleic acid molecule.

15           The kits described herein can include a buffering agent, a preservative, or a polypeptide stabilizing agent. The kits can also include components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate), and/or a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

20           The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted KIF18A expression or activity. “Unwanted” expression or activity can cause pain or deregulated cell proliferation. A disease or disorder associated with misexpressed or aberrant or unwanted KIF18A expression or activity can be a proliferative disorder. A proliferative disorder is a disorder characterized by irregularities in cell division, such as a cancer, psoriasis, or atopic dermatitis. For example, a cancer can be a melanoma, prostate cancer, cervical cancer, breast cancer, colon cancer, or sarcoma. The cancer can be a leukemia. The diagnostic methods described herein can be used to identify a tumor cell, hyperproliferative cell, or neoplastic cell, which all have the capacity for autonomous growth, *i.e.*, an abnormal state or condition



characterized by rapidly proliferating cell growth. The methods can be used to detect cancerous growths or oncogenic processes, or metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The methods can be used to detect malignancies in any organ system, including, but not limited to, the lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract. The methods can also be used to detect adenocarcinomas, which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, which can include, for example, malignant tumors composed of carcinomatous and sarcomatous tissues.

A disease or disorder associated with misexpressed or aberrant or unwanted KIF18A expression or activity can be an autoimmune disorder, such as rheumatoid arthritis. Thus, the diagnostic methods described herein can identify subjects having, or at risk for developing, an autoimmune disorder. Other rheumatic diseases that can be associated with aberrant or unwanted KIF18A expression or activity include systemic lupus erythematosus, Sjögren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, and Reiter's syndrome. Other autoimmune diseases include autoimmune lymphoproliferative syndrome (ALPS); autoimmune disease of the central nervous system, such as multiple sclerosis, myasthenia gravis, and encephalomyelitis; atopic dermatitis; inflammatory bowel disease (*e.g.*, ulcerative colitis or Crohn's Disease).

Methods relating to the invention can be used to detect a disease or disorder associated with aberrant or unwanted KIF18A expression or activity. For example, according to one method, a test sample can be obtained from a subject, and the KIF18A polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) of the sample can be evaluated. For example, the level (*e.g.*, the presence or absence) of KIF18A polypeptide or nucleic acid in the sample can be diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted KIF18A expression or activity. As used herein, a "test sample" refers to a

biological sample obtained from a subject of interest, including a biological fluid (*e.g.*, serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered a compound (*e.g.*, an agonist, antagonist, peptidomimetic, protein, polypeptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted KIF18A expression or activity. For example, such methods can be used to determine whether a compound can be administered to a subject to effectively treat a cell proliferation disorder.

The methods described herein can be used to detect genetic alterations in a *KIF18A* gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in KIF18A protein activity or nucleic acid expression, such as a proliferation disorder. For example, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a KIF18A protein, or the mis-expression of the *KIF18A* gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of the following: 1) a deletion of one or more nucleotides from a *KIF18A* gene; 2) an addition of one or more nucleotides to a *KIF18A* gene; 3) substitution of one or more nucleotides in a *KIF18A* gene; 4) a chromosomal rearrangement of a *KIF18A* gene; 5) an alteration in the level of a messenger RNA transcript of a *KIF18A* gene; 6) aberrant modification of a *KIF18A* gene, such as the methylation pattern of the genomic DNA; 7) the presence of a non-wildtype splicing pattern of a messenger RNA transcript of a *KIF18A* gene; 8) a non-wildtype level of a KIF18A protein; 9) allelic loss of a *KIF18A* gene; and 10) inappropriate post-translational modification of a KIF18A protein.

A genetic alteration can be detected with a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the *KIF18A* gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a *KIF18A* gene under conditions such that hybridization and amplification of the *KIF18A* gene (if present) occurs, and detecting the presence or absence of an amplification product or detecting the size of the amplification product

and comparing the length to a control sample. PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein. Alternative amplification methods are described above and can be used in a preliminary amplification step, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

Mutations in a *KIF18A* gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA can be isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes determined, such as by gel electrophoresis, and compared. Differences in fragment length sizes between sample and control DNA can indicate mutations in the sample DNA. Moreover, the use of sequence-specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

Genetic mutations in a *KIF18A* gene can be identified by hybridizing sample and control nucleic acids (*e.g.*, DNA or RNA) to two-dimensional arrays, such as chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses. For example, the arrays can contain hundreds or thousands of oligonucleotides probes (Cronin *et al.*, *Human Mutation*, 7:244-255, 1996; Kozal *et al.*, *Nature Medicine*, 2:753-759, 1996). In one alternative, genetic mutations in *KIF18A* can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* (*supra*). According to this method, a first hybridization array of probes can be used to scan through long stretches of DNA in a test sample and control sample to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

Any of a variety of sequencing reactions known in the art can be used to directly sequence the *KIF18A* gene and detect mutations by comparing the sequence of the sample

*KIF18A* with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays (Biotechniques 19:448, 1995), including sequencing by mass spectrometry.

Other methods for detecting mutations in the *KIF18A* gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.*, *Science*, 230:1242, 1985; Cotton *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:4397, 1988; and Saleeba *et al.*, *Methods Enzymol.*, 217:286-295, 1992).

The mismatch cleavage reaction employs one or more polypeptides that can recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in *KIF18A* cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.*, *Carcinogenesis*, 15:1657-1662, 1994; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in *KIF18A* genes. For example, single strand conformation polymorphism (SSCP) can be used to detect differences in electrophoretic mobility between mutant and wildtype nucleic acids (Orita *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:2766, 1989, see also Cotton, *Mutat. Res.* 285:125-144, 1993; and Hayashi, *Genet. Anal. Tech. Appl.* 9:73-79, 1992). Single-stranded DNA fragments of sample and control *KIF18A* nucleic acids can be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments can be labeled or detected with labeled probes. The sensitivity of the assay can be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double-stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.*, *Trends Genet.*, 7:5, 1991).

The movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.*, *Nature*, 313:495, 1985). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a

temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner, *Biophys. Chem.*, 265:12753, 1987).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.*, *Nature*, 324:163, 1986; Saiki *et al.*, *Proc. Natl Acad. Sci USA*, 86:6230, 1989).

Alternatively, allele-specific amplification technology that depends on selective PCR amplification can be used to detect nucleotide polymorphisms. Oligonucleotides used as primers for specific amplification can carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.*, *Nucleic Acids Res.*, 17:2437-2448, 1989) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner, *Tibtech*, 11:238, 1993). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.*, *Mol. Cell Probes*, 6:1, 1992). It is anticipated that in certain embodiments, amplification can also be performed using Taq ligase for amplification (Barany, *Proc. Natl. Acad. Sci. USA*, 88:189, 1991). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein can be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which can be conveniently used, *e.g.*, in clinical settings to diagnose subjects exhibiting symptoms or family history of a disease or illness involving a *KIF18A* gene, or to assess the effect of treatment with a pharmaceutical composition of the method on KIF18A activity. For example, a subject can come from a family with a history of a proliferative disorder, such as cancer (*e.g.*, any of the types of cancers described herein) or psoriasis or atopic dermatitis. A subject can alternatively, or in addition, come from a family with a history of autoimmune disease, such as rheumatoid arthritis or any of the other autoimmune disorders described herein. A patient who experiences any symptoms of a proliferative or autoimmune disorder can receive or perform the methods of any of the kits described herein.

### Pharmaceutical Compositions

Therapeutic nucleic acids, polypeptides, antibodies, small non-organic molecules and small non-nucleic acid organic molecules that modulate the activity or expression of KIF18A (also referred to herein as "modulating agents" or "active compounds") can be incorporated into pharmaceutical compositions. Such compositions typically include the active compound and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous; oral (*e.g.*, inhalation); transdermal (topical); transmucosal; and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ), or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage, and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and

liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent, such as aluminum monostearate or gelatin, which delays absorption.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives.

5 Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the modulating agents are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal  
10 delivery.

In one embodiment, the modulating agents are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid,  
15 collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to  
20 those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired  
25 therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic  
30 index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should



be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, for example about 0.01 to 25 mg/kg body weight, about 0.1 to 20 mg/kg body weight, about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The polypeptide can be administered one time per week for between about 1 to 10 weeks, for example, between 2 to 8 weeks, between about 3 to 7 weeks, or for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or a series of treatments.

For antibodies, the dosage can be about 0.1 to 100 mg, *e.g.*, 10 to 60 mg/kg, or 20 to 40 mg/kg. If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method

for lipidation of antibodies is described by Cruikshank *et al.* (*J. Acquired Immune Deficiency Syndromes and Human Retrovirology*, 14:193, 1997).

Compounds that modulate expression or activity of a KIF18A polypeptide or nucleic acid can be included in the pharmaceutical compositions described herein. A compound can, for example, be a small molecule. Such small molecules include, but are not limited to, polypeptides, peptidomimetics (*e.g.*, peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1  $\mu\text{g/kg}$  to about 500 mg/kg, about 100  $\mu\text{g/kg}$  to about 5 mg/kg; or about 1  $\mu\text{g/kg}$  to about 50  $\mu\text{g/kg}$ ). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher can, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin

D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response. The drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety can be a polypeptide possessing a desired biological activity. Such polypeptides can include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:3054-3057,1994). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### Methods of Treatment

Described herein are methods, including prophylactic and therapeutic methods, of treating a subject having or at risk for developing (or susceptible to) a disorder associated with aberrant or unwanted KIF18A expression or activity. Aberrant or unwanted expression or activity can refer to overexpression or increased KIF18A activity, or a loss or decrease in KIF18A expression or activity. In one aspect, the invention provides methods for preventing in a subject, a disease or condition associated with increased KIF18A expression or activity, by administering to the subject a compound that inhibits KIF18A expression or at least one KIF18A activity. A subject having an increased KIF18A expression or activity may have, *e.g.*, a proliferative disorder, such as psoriasis or a cancer. Also described herein are methods for preventing in a subject, a disease or condition associated with decreased KIF18A expression or activity, by administering to the subject a KIF18A nucleic acid or polypeptide, or a compound that stimulates or increases KIF18A expression or at least one KIF18A activity. Decreased KIF18A expression or activity may result in diseases or disorders resulting from inefficient cell replication, *e.g.*, disorders characterized by inefficient wound healing or infertility.

Subjects having or at risk for a disease that is caused or contributed to by aberrant or unwanted KIF18A expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic compound can occur prior to the manifestation of symptoms characteristic of a KIF18A aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the desired effect of a KIF18A target compound, for example, a KIF18A agonist or KIF18A antagonist compound can be used for treating the subject. The appropriate compound can be determined based on screening assays described herein.

Some disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products can bring about the amelioration of disorder symptoms.

As discussed above, KIF18A disorders can be ameliorated by inhibiting the expression or activity of target gene products. For example, compounds, such as a compound identified using an assay described herein, that exhibit negative modulatory activities, can be used in accordance

with the described treatment methods to prevent and/or ameliorate symptoms of a KIF18A disorder. Such molecules can include, but are not limited to polypeptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')<sub>2</sub> and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Antisense, siRNA, and ribozyme molecules that inhibit expression of the target gene can also be used to reduce the level of *KIF18A* gene expression, thus effectively reducing the level of gene activity. Still further, triple helix molecules can be utilized in reducing the level of *KIF18A* gene activity. These molecules are described in detail above.

It is possible that the use of antisense, siRNA, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix or siRNA) and/or translation (antisense, ribozyme, or siRNA) of mRNA produced by a normal *KIF18A* allele, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express KIF18A polypeptides exhibiting normal polypeptide activity can be introduced into cells via a gene therapy method.

Another method by which nucleic acid molecules can be utilized in treating or preventing a disease characterized by *KIF18A* expression includes the use of aptamer molecules specific for a KIF18A polypeptide. Aptamers are nucleic acid molecules having a tertiary structure that permits them to specifically bind to polypeptide ligands (see, *e.g.*, Osborne *et al.*, *Curr. Opin. Chem. Biol.*, 1:5-9, 1997; and Patel, *Curr. Opin. Chem. Biol.*, 1:32-46, 1997). Since nucleic acid molecules can, in many cases, be more conveniently introduced into target cells than therapeutic polypeptide molecules, aptamers offer a method by which KIF18A polypeptide activity can be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for a KIF18A polypeptide and that reduce KIF18A polypeptide activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of a KIF18A disorder. For a description of antibodies, see the "Antibodies" section above.

In circumstances wherein injection of an animal or a human subject with a KIF18A polypeptide or epitope for stimulating antibody production is harmful to the subject, or is

otherwise undesirable, it is possible to generate an immune response against KIF18A through the use of anti-idiotypic antibodies (see, for example, Herlyn, *Ann. Med.*, 31:66-78, 1999; and Bhattacharya-Chatterjee and Foon, *Cancer Treat. Res.*, 94:51-68, 1998). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-idiotypic antibodies, which should be specific to the KIF18A polypeptide. Vaccines directed to a disease characterized by *KIF18A* expression can also be generated in this fashion.

In instances where the KIF18A target antigen is intracellular and whole antibodies are used, internalizing the antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, polypeptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (see *e.g.*, Marasco *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:7889-7893, 1993).

Compounds identified as inhibitors of KIF18A expression, synthesis, and/or activity can be administered to a subject at therapeutically effective doses to prevent, treat, or ameliorate KIF18A-mediated disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of a disorder. For example, a proliferative disorder, such as a cancer, is a KIF18A-mediated disorder that can be treated with a therapeutically effective dose of an identified compound. It is understood that the specific dose level for any particular subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include oral or parenteral (*e.g.*, intravenous, intradermal, subcutaneous, inhalation, transdermal (topical), transmucosal, and rectal administration (*supra*)). The specific KIF18A disease or disorder to be treated can direct an appropriate route of administration. For example, a subject having a wound healing disorder

due to insufficient KIF18A expression or activity, can be administered a topical solution, such as a cream or lotion, containing KIF18A or a modulating agent that increases KIF18A expression or activity.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, such as for determining the LD50 and the ED50 as described above in the Pharmaceutical Compositions section.

Another aspect of the invention pertains to methods of modulating KIF18A expression or activity for therapeutic purposes. Accordingly, in one embodiment, the modulatory method of the invention involves contacting a cell with KIF18A or compound that modulates one or more of the activities of KIF18A polypeptide activity associated with the cell. A compound that modulates KIF18A protein activity can be a compound as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring target molecule of a KIF18A polypeptide (*e.g.*, a microtubule), a KIF18A antibody, a KIF18A agonist or antagonist, a peptidomimetic of a KIF18A agonist or antagonist, or other small molecule (*e.g.*, a small inorganic molecule).

A compound described herein may stimulate one or more KIF18A activities. Examples of such stimulatory agents include active KIF18A polypeptide and a nucleic acid molecule encoding KIF18A. Alternatively, the compound may inhibit one or more KIF18A activities. Examples of such inhibitory compounds include antisense *KIF18A* nucleic acid molecules, anti-KIF18A antibodies, and KIF18A inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the compound) or, alternatively, *in vivo* (*e.g.*, by administering the compound to a subject). As such, methods are described for the treatment of an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a KIF18A polypeptide or nucleic acid molecule. One such method involves administering a compound (*e.g.*, a compound identified by a method, such as a screening assay, described herein), or combination of compounds that can modulate (*e.g.*, upregulate or downregulate) KIF18A expression or activity. In addition, or in an alternative, a KIF18A polypeptide or nucleic acid molecule can be administered as a therapy to compensate for reduced, aberrant, or unwanted KIF18A expression or activity.

Stimulation of KIF18A activity can be desirable in situations in which KIF18A is abnormally downregulated and/or in which increased KIF18A activity is likely to have a beneficial effect. Likewise, inhibition of KIF18A activity can be desirable in situations in which

KIF18A is abnormally upregulated and/or in which decreased KIF18A activity is likely to have a beneficial effect.

### Uses

5           Methods of identifying a compound that modulates the expression or activity of a KIF18A gene or polypeptide are described herein. For example, one method includes contacting the compound with a KIF18A polypeptide and evaluating the ability of the compound to interact with (e.g., to bind or form a complex with) the subject KIF18A polypeptide. Such methods can be performed *in vitro*, such as in a cell free system, or *in vivo*, such as in a two-hybrid interaction  
10       assay. Such methods can be also be used to identify naturally occurring molecules that interact with a subject KIF18A polypeptide. They can also be used to find natural or synthetic inhibitors of a subject KIF18A polypeptide. Screening methods are discussed in more detail above.

          Antibodies described herein can be used in one or more of the following methods without limitation: a) screening assays; b) predictive medicine (e.g., diagnostic assays and monitoring  
15       clinical trials); and c) methods of treatment (e.g., therapeutic and prophylactic). More specifically, the anti-KIF18A antibodies of the invention can be used to detect and isolate related kinesin-like polypeptides, regulate the bioavailability of KIF18A and related polypeptides, and modulate the activity of KIF18A and related polypeptides.

          The therapeutic agents described herein can target a cell (or cells) of interest, such as a  
20       tumor cell, in the case of a cancer, or a cell of a joint in the case of rheumatoid arthritis. For example, a therapeutic nucleic acid molecule, such as an siRNA or antisense nucleic acid, can be conjugated to a ligand that binds a receptor presented on the surface of a target cell, such as a cancer cell. Alternatively, a therapeutic nucleic acid molecule can be conjugated to an antibody that recognizes a ligand present on a target cell. For example, an antibody that binds c-erbB-2,  
25       CO17-1A, MUC-1, or LewisY antigen can be conjugated to a therapeutic agent to treat breast cancer cells (Braun *et al.*, *Int. J. Cancer* 84:1-5, 1999). In yet another alternative, a therapeutic polypeptide can be a fusion polypeptide, wherein the therapeutic amino acid sequence is fused to an amino acid sequence that can bind a receptor or ligand on the surface of a target cell.

          The new methods and compounds (including antibodies) described herein can be used as  
30       an insecticide, such as by targeting KLP67A. For example, an antibody or compound that inhibits KLP67A gene expression or polypeptide activity can be ingested by an insect, which can



result in death or sterility of the insect. In another alternative, an agent that targets KLP67A can be distributed by a viral vector. For example, a virus carrying a nucleic acid encoding an agent (such as a dsRNA or antisense RNA) that targets KLP67A expression or activity can be used to infect insects.

5           The invention is further illustrated by the following examples, which should not be construed as further limiting.

## **EXAMPLES**

### **Example 1: Materials and Methods for Analysis of the Role of**

#### ***Drosophila KLP67A* in Mitosis**

10           KLP67A is the *Drosophila* ortholog of KIF18A (Miki *et al.*, *Proc. Natl. Acad. Sci. USA*, 98:7004-7011, 2001). Thus an analysis of the role of KLP67A in the genetically tractable model organism *Drosophila melanogaster*, can provide insight to the role of KIF18A in vertebrate cells, such as mammalian cells (*e.g.*, human cells).

#### *Vectorette screen for P insertions at KLP67A*

15           The procedure of Eggert *et al.* (*Genetics*, 149:1427-1434, 1998) was followed with the following modifications to recover *P* element insertions in *KLP67A*. As a source of *P*, the strain *l(3)036912* was used as a source of *P* (Deak *et al.*, *Genetics*, 147:1697-1722, 1997), since we had  
20           molecularly mapped the *P* element in this strain to 10kb from the 3' end of the *KLP67A* gene by plasmid rescue. Animals of the genotype *w<sup>-</sup>; l(3)036912* were crossed to a strain containing the immobilized source of *P* transposase, *P[Δ2-3]*. Resulting male progeny with the genotype *w<sup>-</sup>; l(3)036912/P[Δ2-3]* were then crossed to *w<sup>-</sup>; TM6B* females. To screen for insertions in *KLP67A*, DNA was prepared from pools of 35-50 *w<sup>+</sup>* progeny and used as a template for vectorette  
25           mediated inverse PCR as described (Eggert *et al.*, *Genetics*, 149:1427-1434, 1998). To determine the precise insertion site of a potential *P* element in *KLP67A*, PCR reactions were performed with genomic DNA using a *P* element-specific primer (5'-  
CCACCTTATGTTATTTTCATCATG-3') and a *KLP67A*-specific primer (5'-  
CCTTGAATCGCACTCCAATGC-3'). The resulting 900 bp DNA fragment was purified and  
30           sequenced using these same primers.

### Screen for local *P* transpositions

Animals of the genotype  $w^+$ ; *EP(3)3516/TM6B* were crossed to a strain containing the immobilized source of *P* transposase, *P[Δ2-3]*. Resulting male progeny with the genotype  $w^-$ ; *EP(3)3516/[Δ2-3]* were then crossed as single pair matings to  $w^-$ ; *Df(3L)29A6/TM6B* female. To  
 5 screen for local hops, DNA was prepared from pools of progeny and 200 mutagenized lines were screened by PCR with primers specific to *P* and internal to *KLP67A* as described above. A size change in the PCR product or its absence indicated a putative deletion.

### Construction of rescue construct *Pwum*<sup>2</sup>[*myc*:*KLP67A*]

10 A *KLP67A* cDNA was cloned into the BamH I/EcoR I sites of the *P* element transformation vector *Pwum*<sup>2</sup> (Heck *et al.*, *J. Cell Biol.*, 123:665-679, 1993) that allowed *KLP67A* to be expressed as a fusion protein with a myc epitope at the amino terminus. Germ line transformants with chromosome II insertions of *Pwum*<sup>2</sup>[*myc*:*KLP67A*] were recovered and kept as homozygotes. *Pwum*<sup>2</sup>[*myc*:*KLP67A*] was also put into the background of  
 15 *Df(3L)29A6/TM6B* (A.T.C. Carpenter unpublished data). For rescue crosses animals of the genotype  $w^-$ ; *Pwum*<sup>2</sup>[*myc*:*KLP67A*]; *Df(3L)29A6/TM6B* were crossed to *KLP67A*<sup>322b24</sup>/*TM6B* and the resulting progeny which were  $w^-$ ; *Pwum*<sup>2</sup>[*myc*:*KLP67A*]; *Df(3L)29A6/KLP67A*<sup>322b24</sup> were tested for male fertility.

### RT-PCR analysis of insertion lines

20 mRNA was prepared from both mutant and wildtype testes and cDNA was then synthesized using a Qiagen RT-PCR kit (Valencia, CA). To detect transcription from *KLP67A*, a primer specific to the middle of *P[lacW]* (5'-CACCCAAGGCTCTGCTCCCACAAT-3') (SEQ ID NO:5) together with a *KLP67A* reverse primer specific to the coding sequence (5'-  
 25 CCCACATCGAATTTGCGC-3') (SEQ ID NO:6) were used to amplify the *KLP67A* cDNA. The resulting PCR product was then sequenced to verify that the transcript did indeed contain sequences encoded by the *P[lac]* element.

### Immunofluorescence analysis of male sterile mutations

30 Testes were dissected and fixed according to Cenci *et al.* (*J. Cell Sci.*, 107:3521-3534, 1994) for  $\alpha$ -tubulin and either centrosomin or myosin II immunostaining, and to Gunsalus *et al.*

(*J. Cell Biol.*, 131:1234-1259, 1995) for  $\alpha$ -tubulin immunostaining followed by actin staining with phalloidin. Slides were rinsed several times in phosphate buffered saline (PBS) and then incubated overnight at 4°C with anti-centrosomin (Li and Kaufman, *Cell* 85:585-596, 1996) or anti-myosin II (provided by Chris Field, Harvard University; Foe *et al.*, *Development*, 127:1767-1787, 2000) rabbit primary antibodies diluted in PBS. Primary antibodies were detected by a 2 hour incubation at room temperature with Cy3 conjugated anti-rabbit IgG (Harlan Sera-Lab; Leicestershire, England) diluted 1:30 in PBS. Slides were then incubated for 1 hour at room temperature with a monoclonal anti  $\alpha$ -tubulin antibody (Amersham; Piscataway, NJ) diluted 1:50 in PBS and then with FITC-conjugated sheep-anti-mouse IgG diluted 1:20 (1 hour at room temperature; Jackson ImmunoResearch Laboratories; West Grove, PA). For actin plus tubulin staining, testes were first immunostained for  $\alpha$ -tubulin as described and then incubated with rhodamine-phalloidin (Molecular Probes; Eugene, OR) as described in Gunsalus *et al.* (*J. Cell Biol.*, 131:1243-1259, 1995). All preparations were mounted with Vectashield H-1200 with DAPI (Vector Laboratories; Burlingame, CA) and examined with an AxioPlan (ZEISS) microscope equipped with a 50W mercury lamp for epifluorescence and with a cooled charge-coupled device (CCD; Biomedical Photometrics Inc.; Waterloo, Ontario, Canada). Grayscale digital images were collected using IP Lab Spectrum software and then converted to Photoshop 5.0 format, pseudocolored and merged.

### *Live confocal imaging*

For time-lapse confocal microscopy, early embryos (0-2 hours) were manually dechorionated, transferred to adhesive-coated coverslips, and briefly dehydrated to accommodate the addition of injected material. The dehydrated embryos were then covered with halocarbon oil and transferred to the stage of the confocal microscope. A fluorescent conjugate of tubulin (tetramethylrhodamine-tubulin; Cytoskeleton Inc.; Denver, CO) was then microinjected into the embryos at a concentration of 5  $\mu$ g/mL. The behavior of the labeled tubulin was visualized directly using a Leica TCS-SP laser-scanning confocal microscope as described in Theurkauf and Heck (*Methods Cell Biol.*, 61:317-346, 1999).

### *Cell culture and transfection of KLP67A dsRNA*

*Drosophila* cultured cells (DL2) were grown at room temperature in Schneider's *Drosophila* medium supplemented with 10% fetal bovine serum and 1x penicillin streptomycin solution. DL2 cells, derived from the *Drosophila* Schneider cell line, SL2, were provided by  
 5 Norbert Perrimon, Harvard Medical School. For transfection, cells were resuspended and cultured at  $2 \times 10^5$  cells/mL in a 24 well dish with and without coverslips for 24 hours before transfection. Cells were transfected using standard calcium phosphate methods with 5  $\mu$ g *KLP67A* dsRNA (FIG. 21; SEQ ID NO:14) per well in 1 mL medium. After 16-18 hours of transfection, cells were washed with fresh medium and cultured for another 24 hours before  
 10 staining for immunofluorescence or assaying by RT-PCR.

To prepare for immunofluorescence staining, cells were fixed in methanol and immunostained for  $\alpha$ -tubulin (Sigma; St. Louis, MO), centrosomin (Heuer *et al.*, *Development*, 121:3861-76, 1995; Li and Kaufman, *Cell*, 85:585-96, 1996; provided by T. Kaufman, Indiana University; Bloomington, IN) and DAPI (Sigma; St. Louis, MO).

For synthesizing dsRNA, a 500 bp fragment of *KLP67A* was PCR amplified using gene-specific primers, 5'-T7-AGTACGGCCGTATAATGTCCGTG-3' (SEQ ID NO:7) and 5'-T7-CCACTGACCACCACGCCATTG-3' (SEQ ID NO:8) to allow *in vitro* transcription from both strands of the 500 bp *KLP67A* PCR fragment. For synthesizing *KLP61F* dsRNA (FIG. 20; SEQ ID NO:13), gene-specific primers, 5'-T7-GACGGGCACAGGGAAGACCCAC-3' (SEQ ID  
 15 NO:9) and 5'-T7-TCCCTTTTCATTCCAGCCTTGG-3' (SEQ ID NO:10) were used. An Ambion MEGAscript™ T7 kit (cat# 1334; Austin, TX) was used for the *in vitro* transcription reaction. The RNA was precipitated with lithium chloride and dissolved in water. It was annealed by heating at 1% agarose gel and quantified by UV spectrophotometry. For RT-PCR, the RNA was isolated from cells using a Qiagen RNeasy® Mini Kit (Qiagen Inc.; Valencia, CA)  
 20 and amplified employing Qiagen One-Step RT-PCR Kit with *KLP67A* gene-specific primers (5'-CGAAAACCAAACAAGAGC-3' (SEQ ID NO:11) and 5'-CCCACATCGAATTTGCGC-3' (SEQ ID NO:12)).

### **Example 2: Isolation of Mutations in the *KLP67A* Gene**

30 To examine the role of the kinesin-like protein *KLP67A* in cell proliferation, a vectorette-mediated PCR screen was used to isolate mutations in the *Drosophila KLP67A* gene (Eggert *et*

*al.*, *Genetics*, 149:1427-1434, 1998). To conduct this screen, pre-existing *P* element insertions in the 67A region were molecularly mapped to the cloned *KLP67A* interval. Plasmid rescue of several of the previously identified *P* element lines at 67A (Deak *et al.*, *Genetics*, 147:1697-1722, 1997) revealed that two of these insertions were close to the 3' end of the *KLP67A* gene. Strain *l(3)036912* contains a *P* element within 6 kb of the gene, and strain *l(3)049508* contains an element within 11 kb of the 3' end of the gene. In two separate vectorette screens, these elements were mobilized and insertions in *KLP67A* were assayed by PCR of genomic DNA that had been restriction digested and ligated to a vectorette fragment (Eggert *et al.*, *Genetics*, 149:1427-1434, 1998). For each screen, pooled samples of genomic DNA, prepared from 1000 mutagenized lines of flies, were used for the PCR reactions and then the products were probed with a *KLP67A* cDNA (GenBank Accession No. NM\_079268; SEQ ID NO:4; FIG. 19).

Several pools of DNA contained a *P* element insertion at the same site indicating that *KLP67A* contains a "hot spot" for *P* element integration. DNA sequence analysis of the PCR fragment resulting from positive line, *P [lac w]15D*, revealed that the hotspot is 130 bp upstream from the transcription start of *KLP67A*. Upon completion of this screen, a *P* element insertion, *EP(3)3516*, was identified in this same location from the Rorth collection (Rorth, *Genetics*, 93:12418-12422, 1996) during the *Drosophila* genome sequencing project (Spradling *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:10824-10830, 1995). These insertions were found to be homozygous viable. They also had no phenotype when made heterozygous over the deficiency *Df(3L)29A6* that lacks the *KLP67A* sequence.

A second type of PCR screen for "local hops" (Tower *et al.*, *Genetics*, 133:347-359, 1993) as well as *P* transposition associated aberrations was conducted using the *EP(3)3516* element (Rorth, *Proc. Natl. Acad. Sci.*, 93: 12418, 1996; the sequence of the EP element was compiled at the internet-based database, Flybase (Gelbart, W.M., 1994. Personal communication), and has the designation Fbmc0001297). The element was mobilized in males and the F2 generation was screened for eye color changes including white eyes. Potential mutations were assayed by PCR using a *P* element-specific primer as well as a *KLP67A* gene-specific primer. PCR reactions were examined for either an altered size fragment or an absence of product. Twenty potential mutations were isolated from 200 lines screened. DNA sequence analysis of several of these lines revealed that they contained small deletions of 35 bp or fewer

within the promoter region of *KLP67A*. One of these lines, designated *KLP67A*<sup>322b24</sup>, was then used for further characterization. It was determined by RT-PCR analysis that *KLP67A* transcription begins within the *rosy* (*ry*) gene of the *P* element, which was inserted 130 bp upstream of the transcription start site for *KLP67A* (155 bp upstream of the start codon for the *KLP67A* ORF). The insertion corresponds to bp position 9,317,954 of GenBank Accession Number NT\_037436.

### **Example 3: The Meiotic Phenotype of *KLP67A* Mutants**

*KLP67A*<sup>322b24</sup>/*Df(3L)29A6* flies were viable and did not exhibit a visible phenotype.

However, males of this genotype were either sterile or semi-sterile. Testes from *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* males were dissected, squashed, and examined cytologically for the presence of meiotic defects. As shown in FIGs. 1-11, a number of microtubule-related defects were observed throughout all stages of the first meiotic division. First, in 89% (n = 74) of late prophase images (stage M1 according to Cenci *et al.*, *J. Cell Sci.*, 107:3521-3534, 1994), the asters were not properly positioned at the opposite sides of the nucleus as in wildtype (see FIG. 1) but ectopically localized in the cytoplasm and close to each other, as demonstrated in FIG. 2; occasionally, centrosomes and their associated asters appeared to detach from the poles, giving rise to asters that freely float in the cytoplasm (see FIG. 3). In other cases, evidence failed cytokinesis in a gonial division could be found in tetraploid spermatocytes. These spermatocytes also demonstrated ectopically positioned asters (see FIG. 4).

Despite the defect in aster localization and separation, most *KLP67A* primary spermatocytes (96%; n = 52) appeared to progressively assemble a bipolar spindle, which could mediate the formation of a metaphase plate (FIG. 7, cell indicated by arrowhead) and to proceed through anaphase (FIG. 7, arrow). FIG. 5 shows a normal spermatocyte isolated from the testes of wildtype *Drosophila* in the metaphase I stage of meiosis. The spermtocyte is stained for  $\alpha$ -tubulin, centrosomin, and DNA. The whiter spots represent the centrosomes. FIG. 6 shows a normal spermatocyte in the anaphase I stage of meiosis. Mutant metaphase and anaphase spindles both displayed astral MTs that appeared to be longer than their wildtype counterparts (see FIG. 7). In addition, in 12% of anaphase images (n = 25) the two daughter nuclei were of different sizes, suggesting aberrant chromosome segregation (FIG. 7, arrow). Telophase I spindles of *KLP67A* spermatocytes were even more irregular. FIG. 8 is a photomicrograph of

wildtype spermatocytes in telophase I, and FIG. 9 is a photomicrograph of *KLP67A* mutant spermatocytes in telophase I. In 60% ( $n = 60$ ) of telophase images, astral MTs were longer in mutant than in wildtype cells, and the central spindle was either absent or much less dense than that seen in normal cells (compare FIGs. 8 and 9). An examination of secondary spermatocytes undergoing the second meiotic division revealed that only 18% of late prophase images ( $n = 60$ ) exhibited ectopic aster localization. However, cells in subsequent stages of meiosis II exhibited the same defects observed in primary spermatocytes undergoing meiosis I. Interestingly, 47% of ana-telophase II images ( $n = 132$ ) displayed two spindles within the same cytoplasm (see FIG. 11), suggesting a failure of cytokinesis during the first meiotic division. In these “double” secondary spermatocytes, astral MTs were also long, so that MTs from asters of different spindles often overlapped, resulting in a highly disorganized spindle architecture (FIG. 11). Compare the mutant cells of FIG. 11 to the wildtype spermatocytes in FIG. 10, also in telophase II of meiosis.

To characterize the cytokinetic phenotype of *KLP67A* mutants, spermatocytes were stained for both myosin II and F actin, two well-known components of the contractile ring. By these studies, wildtype spermatocytes exhibited a robust central spindle and a clear acto-myosin ring. Mutant telophases that had normal central spindles also exhibited a regular contractile ring. However, mutant telophases with a poorly organized central spindle displayed a diffuse actin and myosin staining at the cleavage furrow rather than the typical tight band seen in wildtype cells. These results indicated that *KLP67A* telophases having a defective central spindle are unable to assemble a contractile ring and to undergo cytokinesis.

Spermatid morphology of *KLP67A* mutant spermatocytes was observed to determine the effect of the mutant-induced spindle defects. In wildtype spermatocytes, both chromosomes and mitochondria were equally partitioned between the two daughter cells at each meiotic division. After completion of meiosis, the mitochondria fused to form a conglomerate called the nebenkern. Thus, each wildtype spermatid contained a round nucleus associated with a single nebenkern of similar size. Defects in chromosome segregation resulted in differently sized nuclei (Gonzalez *et al.*, *Genet. Res.*, 54:205-212, 1989), whereas failures in cytokinesis gave rise to spermatids having a large nebenkern associated with either two or four normal-sized nuclei (Fuller, *Spermatogenesis*. In: *The Development of Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, Plainview, N.Y. Vol. I. M. Bate and A.M. Arias, eds., pp. 71-147,

1993). An examination of *KLP67A* mutant spermatids revealed that 41% of these cells (n=254) had large nebenkern associated with two or four nuclei. Twenty-four percent of the nuclei also displayed irregular sizes. Taken together, these observations suggested that *KLP67A* spermatocytes are defective in both chromosome segregation and cytokinesis.

Finally, it should be noted that a few spermatocytes were observed to be polyploid, having four rather than two centrosomes. This finding suggested that *KLP67A* is also required for cytokinesis during the gonial mitoses that precede meiotic division. Both the sterility and the meiotic phenotypes are completely rescued by the *Pwum*<sup>2</sup> [*myc:KLP67A*] transgene (see Example 1).

#### **Example 4: *KLP67A* Maternal Effect on Embryonic Divisions**

Females of the genotype *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* exhibited reduced fertility compared to the parent strains *KLP67A*<sup>322b24</sup>/*TM6B* and *Df(3L)29A6*/*TM6B*. Forty percent of the eggs laid by *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* females hatched to first instar larvae compared to 96% of the eggs laid by a wildtype strain. To determine whether this maternal effect was due to a defect in early blastoderm mitoses, eggs from *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* mothers crossed to wildtype males were collected and used for real time analysis of mitosis in living embryos. Rhodamine labeled tubulin was injected into living embryos and mitosis was allowed to proceed in real time. Time-lapse analysis of MTs showed that both spindle formation and architecture is abnormal through all stages of mitosis.

FIGs. 12-15 are photomicrographs representing the real time analysis of mitosis in the blastoderm of wildtype (FIGs. 14 and 15) and *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* mutant (FIGs. 12 and 13) *Drosophila* embryos. Arrows draw attention to the correlation between incomplete centrosome separation (see FIGs. 12 and 14) and curved banana-shaped spindles (see FIGs. 13 and 15). During prophase, the majority of the centrosomes in the *KLP67A*<sup>322b24</sup>/*Df(3L)29A6* mutant embryos did not complete their migration to the opposite sides of the nucleus (FIG. 12, arrows). The average angle between prophase centrosomes (n=60) was observed to be 145.2° in mutants and 164.2° in wildtype embryos (n = 54). This difference is statistically significant according to the student's T test (P=0.0002). These incompletely separated centrosomes then gave rise to curved banana-shaped spindles (FIG. 13). This centrosome migration defect was correlated with the misshapen spindle phenotype at metaphase in both wildtype and mutant



embryos. However, in mutant embryos the malformation not only occurred more frequently, but it was also exacerbated. The time-lapse video also revealed that the distortion of the normal shape of the spindle occurred at the moment when dynamic MTs appeared to be extending downward to reach the chromosomes. At this precise moment the spindle became distorted and banana-shaped to accommodate the extended MTs emanating from the spindle poles (*infra*). In addition, some prometaphase and metaphase centrosomes detached from the spindle poles, as occurs during meiosis in mutant males. The timing of centrosome separation was also affected by a reduction in *KLP67A*. The period of centrosome migration up until the point of nuclear envelope breakdown lasted for at least 3 minutes; 1 minute longer than in wildtype embryos.

A second important feature of the mutant metaphase spindles was their increased length compared to wildtype spindles. The average pole-to-pole distance within the metaphase spindles (n=60) in mutant embryos was 15.6  $\mu\text{m}$  (+/- 1.5 SD) compared to 10.3  $\mu\text{m}$  (+/- 0.64 SD) in wildtype embryos (n=54). It is important to note that this increased spindle length was observed in all mutant spindles and was not a consequence of the defect in centrosome separation, as even those spindles with normal centrosome positioning were longer than their wildtype counterparts. In addition, in all types of spindles the increase in pole-to-pole length was clearly due to an increase in MT length. These results suggested that the activity of *KLP67A* is required for limiting the length of spindle MTs.

A third important feature of the *KLP67A* mutant phenotype was seen during telophase, when most spindles appeared to be either missing a normal central spindle or to have a greatly reduced number of midzone MTs. Central spindle formation normally occurs during anaphase when the overlapping set of anti-parallel interpolar MTs becomes bundled (Mastronarde *et al.*, *J. Cell Biol.*, 123:1475-1489, 1993). Although areas of MT overlap existed, these MTs were not organized in the characteristic dense lateral arrays that characterize wildtype central spindles. This suggested that the abnormally long, and often curved or bent, astral MTs were not able to interact properly to give rise to the ordered parallel array of central spindle MTs. Since these early blastoderm mitoses did not require MTs to form the pseudo-cleavage furrow (Stevenson *et al.*, *Nature Cell Biology*, 3:68-75, 2001), aberrant spindles were often able to proceed through telophase and two daughter spindles formed in the ensuing divisions. Chromosome behavior was also observed in eggs laid by *KLP67A*<sup>322b24</sup>/*Df*(3L)29A6 females using real time analysis of eggs

injected with OliGreen®. Despite the structural abnormalities observed in the spindles of these eggs, chromosome segregation appeared normal.

To analyze spindle dynamics in *KLP67A* mutant embryos, still images from time-lapse microscopy videos were used to measure the increase in spindle length during the interval between nuclear envelope breakdown ( $t = 0$  seconds) and the appearance of a central spindle (albeit drastically abnormal in the mutant embryo). This period lasted 2 minutes longer in mutant cells (360 seconds) than in wildtype cells (240 seconds). This difference was primarily due to an increase in the amount of time spent in metaphase and anaphase B. During metaphase, the mutant spindles continued to increase in length whereas their wildtype counterparts reached a plateau. In mutant embryos, however, the pause at metaphase (before the final spike in pole separation at anaphase B) was delayed and occurred later than in wildtype. Although wildtype and mutant spindles did not exhibit noticeable differences in the speed of chromosome movement during anaphase A, the mutant spindles took significantly longer to go through anaphase B (90 seconds in the mutants versus 30 seconds in the wildtype). This slowing of anaphase B may be related to the unusual S shaped conformation assumed by mutant spindles during pole separation.

An antibody specific for a *KLP67A* polypeptide (Pereira *et al.*, *J. Cell Biol.*, 136:1081-1090, 1997) was used to assess the effect of the *322b24* mutation on *KLP67A* protein expression. Antibody staining of eggs derived from mutant mothers, as well as Western blot analysis, demonstrated that the *KLP67A*<sup>322b24</sup> allele only mildly affected protein expression levels; the intensity of antibody staining was almost equal to wildtype. Quantitation of Western blot experiments indicated the expression from the *KLP67A*<sup>322b24</sup> allele was about 11% less than expression from the wildtype allele. Together, these results indicate that MT polymerization and, as a result, spindle assembly is extremely sensitive to a slight decrease in the expression level of *KLP67A* during both male meiosis and embryonic mitoses.

These data demonstrate that it is not necessary to fully suppress expression or activity of *KLP67A* or a related gene, *e.g.*, *KIF18A*, to elicit an effect on cellular proliferation. Thus, compounds that partially inhibit expression or activity of a *KLP67A* nucleic acid or polypeptide (or an orthologous nucleic acid or polypeptide, *e.g.*, *KIF18A*) can be useful for inhibiting cellular proliferation.

### **Example 5: RNA Interference of *KLP67A***

A genetic null allele of *KLP67A* would greatly facilitate the functional analysis of this gene. Since a null allele had yet to be successfully generated (strongly suggesting that *KLP67A* is an essential gene), RNA interference (RNAi) was used to create a *KLP67A* null phenocopy.

These experiments were performed using the DL2 *Drosophila* cell line. The flat morphology of these cells allowed a clear view of individual MTs, thereby permitting a reliable evaluation of the effect of *KLP67A* on MT length. Cells were transfected with a 500 bp double-stranded (ds) RNA, designed to target *KLP67A* (see Example 1). Transfected and mock-transfected cells were incubated for two days before they were harvested and fixed for visualization by immunofluorescence. Mitotic images were observed by indirect immunofluorescence. The frequencies of cells in the different stages of mitosis in the treated vs. control cells is recorded in Table 1. It was evident from these data that RNAi caused a mitotic block at metaphase. This suggests that the *KLP67A* RNAi cells were unable to progress to anaphase and telophase due to the activation of the mitotic checkpoint that prevented cells with defective spindles to enter anaphase.

Similar results were observed in a Snyder (SL2) cell line transfected with dsRNA targeted to *KLP67A*.

**Table 1. RNAi of *KLP67A* results in a mitotic arrest**

Transfection Condition	Mitotic Index	# of mitotic images	Prophase %	Prometaphase %	Metaphase %	Anaphase %	Telophase %
Control	3.4 <sup>a</sup>	85	17.6	12.9	52.9	8.2	8.2
<i>KLP67A</i> (RNAi)	13.1 <sup>b</sup>	252	3.6	23.8	69.84	1.5	1.1

DL2 Cells were transfected with *KLP67A* dsRNA (*KLP67A*, RNAi) (FIG. 21; SEQ ID NO:14) or mock transfected (control). The mitotic index is percentage of cells in mitosis, recorded after 2 days. <sup>a</sup>n=2,430; <sup>b</sup>n=1,923.

Although the metaphase arrest of *KLP67A* RNAi cells precluded observation of the entire mitotic process, certain aspects of the phenotype of these cells resembled the *KLP67A* maternal effect observed in blastoderm embryos. The spindles of dsRNA-transfected cells were extremely elongated. The pole-to-pole distance in the control spindles were less than half of that observed in the (RNAi) spindles. These enlarged spindles also showed interpolar MTs that were

abnormally long and curved. In addition, the overall shape of these spindles was often curved as was observed in the blastoderm mitotic divisions (see FIGs. 13 and 15), again indicating incomplete centrosome separation. The metaphase chromosome configuration was also abnormal and a tight metaphase plate configuration was rarely seen.

The observed aberrations were most likely due to an increase in MT stability. To test this, nocodazole was added to control cultures and cultures transfected with *KLP67A* dsRNA (FIG. 21; SEQ ID NO:14) to a final concentration of 15  $\mu$ M. This concentration of nocodazole destabilized MTs and perturbed spindle assembly in control DL2 cells, but only slightly affected spindle architecture and had no effect on astral MT arrays in *KLP67A* depleted cells. This demonstrated that inhibition of *KLP67A* expression or activity (or expression or activity of an ortholog, *e.g.*, KIF18A) can be used to stabilize spindle assembly.

The RNAi phenotype observed in cultured cells corresponded to a strong mutant allele. RT-PCR analysis indicated that the *KLP67A* dsRNA treatment results in a significant decrease in the *KLP67A* mRNA after two days of treatment. Moreover, the RNAi phenotype was not an indirect effect of the mispositioning of the mitochondria since the location of mitochondria relative to the spindle was identical in treated and untreated cells.

#### **Example 6: Double RNA Interference of *KLP61F* and *KLP67A***

The increase in MT length observed in *KLP67A* mutant cells could indicate a requirement for *KLP67A* in the regulation of MT length. Alternatively, *KLP67A* could be required for maintaining normal spindle pole separation, and the increased pole-to-pole distance in *KLP67A* mutant cells could result in an increased MT polymerization. To discriminate between these possibilities, monopolar spindles were examined for abnormal MT elongation in the absence of *KLP67A*. To perform this analysis, DL2 cells were co-transfected with both *KLP61F* dsRNA (FIG. 20; SEQ ID NO:13) and *KLP67A* dsRNA (FIG. 21; SEQ ID NO:14). Previous genetic analyses (Heck *et al.*, *J. Cell Biol.*, 123:665-679, 1993) and antibody injection experiments (Sharp *et al.*, *J. Cell Biol.*, 144:125-138, 1999b) had shown that *KLP61F* depletion prevents centrosome separation, resulting in monopolar spindles. Similarly, treatment of DL2 cells with *KLP61F* dsRNA frequently produced spindles with unseparated asters. These monopolar

spindles were associated with highly condensed chromosomes that appeared to be in metaphase. Transfection of DL2 cells with dsRNA targeting both *KLP61F* and *KLP67A* effectively reduced the expression of both mRNAs and also resulted in monopolar spindles. However, these monopolar spindles displayed a dramatic increase in MT length compared to those observed in cells transfected with *KLP61F* dsRNA alone. In addition, the individual MT fibers of cells transfected with siRNA targeting both *KLP61F* and *KLP67A* had abnormal monopolar spindles. These fibers were not only much longer than those observed in *KLP61F* depleted cells, but they were also bent and curved as those observed in mutant *KLP67A* embryos. The effect of *KLP67A* on MT length was observed as early as prometaphase and in some cases prophase.

#### **Example 7: Subcellular Localization of Human KIF18A**

The localization of human KIF18A protein was determined in human 3T39 fibroblasts. A GFP-tagged *KIF18A* transgene was localized to the ends of microtubules during telophase of mitosis. Immunohistochemical staining using anti-KIF18A antibodies revealed a similar localization pattern. The KIF18A peptide used to raise antisera consists of amino acid residues 492-507 of SEQ ID NO:2 (FIG. 17).

genbank (?) accession no AL136819 and/or SPTREMBL : Q9H0F3 This is consistent with the staining pattern of *KLP67A* in *Drosophila* cells. This also demonstrates a type of cell that can be used to assay test compounds for their ability to alter KIF18A localization.

#### **Example 8: Overexpression of Human KIF18A**

To create a GFP-tagged *KIF18A* construct, the *KIF18A* open reading frame was PCR amplified from cDNA clone DKFZp434g2226 (Resource Center/Primary Database; Berlin, Germany). The 5' ("Forward") primer contained a Xho I restriction site and the 3' ("Reverse") primer contained a BamH I restriction site. Following PCR amplification, the resulting *KIF18A* cDNA was digested with the two enzymes, and the product was cloned into pEGFP-C3 (cat. # 6082-1 Clontech; Palo Alto, CA). This placed the GFP-tagged *KIF18A* construct under the control of a CMV promoter. One half microgram of the plasmid in 1.5  $\mu$ l FuGENE 6 Transfection Reagent (Roche; Indianapolis, IN) was transformed into human 3T39 fibroblast cells. The cells were allowed to recover for two days, before the effect on cell division was monitored by live confocal imaging. These recordings, recorded over 2-4 minute intervals,

revealed that, in living cells, the MTs of monopolar spindle asters appeared to de-polymerizing back to the centrosome. These results suggest that overexpression of *GFP-KIF18A* caused an increased rate of microtubule depolymerization during mitosis, a result that is consistent with the observation that loss of function mutations in the *Drosophila* ortholog *KLP67A* caused a decreased rate of MT depolymerization (*supra*).

Immunofluorescence staining of fixed cells revealed generally disorganized spindles in control and *KIF18A* transfected cells.

The method described can be used in assays to screen for compounds that inhibit KIF18A expression or activity. For example, a test compound is incubated with a cell that overexpresses a KIF18A polypeptide. A decrease in the rate of microtubule depolymerization in the presence of the test compound indicates that the compound inhibits KIF18A expression or activity.

#### **Example 10: Synthesis Of A Human KIF18A Antibody**

An anti-KIF18A antibody was raised against a polypeptide fragment defined by residues 492-507 of SEQ ID NO:2 (FIG. 17). The polypeptide was synthesized by the Molecular Biology Core facility, University of Massachusetts (Worcester, MA), and antibodies were generated in mice.

#### **OTHER EMBODIMENTS**

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.